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High fasting insulin levels are associated with metabolic syndrome in non-diabetic middle-aged and elderly populations: A community-based study in Taiwan

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ABSTRACT

Objectives: We determined the relevance between fasting insulin levels and metabolic syndrome (MetS), and suggested a value of fasting insulin (FI) which can help detect possible comorbidity of MetS in non-diabetic middle-aged and elder adults.

Design: Cross-sectional observational study.

Setting: Community-based investigation in Guishan township of northern Taiwan.

Participants: Our study included adults aged 50 years and above during community health exams between January and October 2014. People with diabetes mellitus were excluded. A total of 321 people were enrolled.

Outcome measures: We divided participants according to tertiles of FI as low, medium, and high levels. Pearson correlation was assessed between insulin level and each of the diagnostic components of metabolic syndrome (MetS-DCs) with adjustment of age. The prevalence of MetS-DCs based on tertiles of insulin levels were studied and analyzed by Cochran-Armitage trend test. The risk for prevalence of MetS in the middle, and high insulin group as compared to the low insulin group were assessed by multivariate logistic regression with adjustments for age, gender, smoking, body mass index, hypertension, and hyperlipidemia. Youden Index was performed for the optimized cut-off value.

Results: Our results showed positive correlation of FI level with systolic blood pressure, waist circumference, fasting plasma glucose and triglyceride levels, while negative correlation was shown with high-density lipoprotein ($p < 0.001$). The prevalence of each MetS-DCs increased as a trend while FI levels increased ($p < 0.001$). OR (95% CI) of MetS was 5.04 (2.15-11.81) for high insulin groups as compared to the low insulin group after adjusting multiple factors ($p < 0.001$). AUC was 0.78, and cut-off value 7.35 $\mu\text{U/mL}$ for FI was obtained (sensitivity: 0.69; specificity: 0.77).

Conclusions: People with increased FI are associated with a higher prevalence of MetS. The

proposed cut-off value of FI may act as a marker for increased risk of MetS in middle-aged and elderly non-diabetic adults.

Keywords: metabolic syndrome, insulin resistance, fasting insulin level

Strengths and limitations of this study:

- This is the first study to explore the relationship between fasting insulin and MetS in the middle-aged and elderly populations of non-diabetic Asian people.
- We offer an easily measured biomarker to identify Chinese middle-aged and elderly with MetS.
- We adjusted many confounding factors to make the results more reliable.
- The recall bias from self-reported lifestyle behaviors are unavoidable.
- The causal relationship between fasting insulin level and MetS can't be demonstrated in our cross-sectional study.

INTRODUCTION

Metabolic syndrome (MetS) is associated with a cluster of unhealthy metabolic risk factors, including abdominal obesity (excess body fat around the waist), glucose intolerance, pre-morbid hypertension, and dyslipidemia¹⁻³. A number of studies have reported that MetS increases the risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and other non-communicable diseases (NCDs)⁴⁻⁷. The rising prevalence of MetS has created a disturbing challenge to personal health⁸⁻¹².

Insulin resistance has long been associated with MetS¹³⁻¹⁶. Basal insulin represents 45%-50% of daily insulin^{17 18}. The fasting insulin level approximates basal insulin^{18 19}, and the level increases as the body mass index (BMI) increases²⁰. A number of studies have shown that elevated fasting insulin levels may predict the development of MetS^{19 21}. We therefore determined the relationships between fasting plasma insulin and MetS in middle-aged and elderly populations. In addition, we also determined whether or not there is a cut-off value for fasting insulin as a predictive factor for the existence of MetS. By determining a cutoff value for fasting insulin, we could educate patients at risk for MetS and recommend aggressive lifestyle modification at an earlier stage.

METHODS

Study subjects

This was an observational and cross-sectional study conducted at Linkou Chang Gung Memorial Hospital in Taoyuan County, Taiwan between January and October 2014. Residents, 50-90 years of age, were eligible for the study. Participants diagnosed with diabetes mellitus were excluded. A total of 321 participants (111 males and 210 females) were ultimately enrolled for analysis. This study was approved by the Institutional Review Board of the hospital and written informed consent was obtained from all of the participants.

before enrollment.

Data collection

We obtained exercise (exercising ≥ 3 times a week or not) and dietary habits (vegetarian or not) from self-administered questionnaires, which also included smoking (current smoker or not) and marital status (currently married or not). Anthropometric data, such as height, weight, waist circumference (WC), and blood pressure were also recorded. The subjects were dressed in light clothing without shoes for weight and height measurements. The BMI was calculated as the weight in kilograms (kg) divided by the height in meters squared (m^2). Waist circumference was measured midway between the inferior margin of the lowest rib and the iliac crest in the horizontal plane while in an upright position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were checked at least 2 times after 5 min of rest while seated. Fasting insulin levels, lipid profile, and fasting glucose were obtained by blood sampling after a 10-h overnight fast. Blood samples were analyzed in the central laboratory of Linkou Chang Gung Memorial Hospital for fasting plasma glucose (FPG), serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), serum triglycerides (TG), and fasting insulin levels.

Defining MetS

MetS was defined by at least 3 of 5 metabolic syndrome diagnostic components (MetS-DCs), according to The Third Report of the National Cholesterol Education Program Expert Panel (NCEP) on Adult Treatment Panel (ATP III) Asian diagnostic criteria²². The five MetS-DCs were as follows: 1) SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg, or the use of anti-hypertensive drugs; 2) decreased serum HDL-C concentration < 40 mg/dl in men and < 50 mg/dL in women, or treatment for dyslipidemia; 3) TG concentration ≥ 150 mg/dl, or on medication for hypertriglyceridemia; 4) hyperglycemia: fasting plasma glucose level ≥ 100 mg/dl, previously diagnosed with diabetes mellitus, or on medication for diabetes mellitus;

and 5) abdominal waist circumference ≥ 90 cm in men or ≥ 80 cm in women.

Statistical Analysis

Subjects were classified in one of three groups according to serum insulin level tertiles as the low, middle, and high insulin groups. Clinical characteristics were expressed as the mean \pm standard deviation (SD) for continuous variables and number (%) for categorical variables. One-way analysis of variance (ANOVA) or a Chi-square test was used to determine p-values for continuous or categorical variables, respectively. Pearson's correlation was performed for each MetS-DC in relation to fasting insulin levels. The Cochran-Armitage trend test was used to evaluate the increasing prevalence of MetS-DCs as a function of insulin level tertile. The low fasting insulin group was designated as the reference group to calculate the ORs of the prevalence of MetS in the middle and high fasting insulin groups. The trend test was used to identify the association between the fasting insulin level with the prevalence of MetS. A receiver operating characteristic curve (ROC) curve was created for fasting insulin as a predictor of MetS. The area under the ROC curve (AUC) was analyzed, and the optimized cut-off point for fasting insulin, sensitivity, and specificity were acquired using the maximal Youden's Index. We used SPSS (version 23.0 for windows), to perform the statistical analysis. Statistical significance was set at a p-value < 0.05 .

RESULTS

A total of 321 individuals, 111 men (34.6%) and 210 (65.4%) women, with a mean age of 63.91 ± 8.32 years, were enrolled in the current study. There were 90 study participants (28%) who met the diagnosis of MetS.

Table 1 shows the characteristics of the study population, which was divided based on the fasting insulin level in $\mu\text{U/mL}$. There were no statistically significant differences in age or gender between the low, middle, and high insulin level groups, while differences did exist with respect to WC, SBP, HDL-C, TG, and the proportion with MetS. Table 2 further shows

the correlation between the fasting insulin level and all MetS-DCs, even after adjusting for age. Fasting insulin was positively correlated with SBP, WC, FPG, and TG, and negatively correlated with HDL-C, as shown in Table 2. Table 3 shows the prevalence of MetS-DCs (hypertension, hyperglycemia, dyslipidemia, and central obesity) according to the insulin level tertiles. The prevalence of MetS-DCs increased as the fasting insulin level increased, as shown by significant p values (Cochran-Armitage trend test). Figure 1 shows that the low insulin level group had a 10% prevalence of MetS, the middle insulin level group had a 21.5% prevalence of MetS, and the high insulin level group had a 53.8% of MetS ($p<0.0001$ [Cochran-Armitage trend test]), suggesting that the prevalence of MetS increased with an increase in fasting insulin levels.

Table 1 General characteristics of the study population based on insulin levels.

Variables	Insulin levels				p value
	Total (n=321)	Low (n=110) (≤ 4.8)	Middle (n=107) (4.9-7.8)	High (n=104) (≥ 7.9)	
Age (year)	63.91 \pm 8.32	64.23 \pm 8.32	64.47 \pm 8.67	63.01 \pm 7.93	0.40
BMI (kg/m ²)	24.36 \pm 3.53	22.41 \pm 3.14	24.41 \pm 2.73	26.37 \pm 3.54	<0.001
Waist circumference (cm)	84.23 \pm 9.51	79.69 \pm 7.57	83.78 \pm 8.63	89.51 \pm 9.65	<0.001
SBP (mmHg)	129.02 \pm 16.59	123.69 \pm 17.36	129.52 \pm 14.51	134.13 \pm 16.20	<0.001
DBP (mmHg)	77.01 \pm 10.90	75.43 \pm 11.80	76.92 \pm 10.03	78.79 \pm 10.60	0.08
ALT (U/L)	21.74 \pm 11.06	18.94 \pm 7.81	20.33 \pm 9.25	26.15 \pm 14.05	<0.001
Creatinine (mg/dL)	0.76 \pm 0.44	0.69 \pm 0.17	0.85 \pm 0.66	0.75 \pm 0.34	0.03
FPG (mg/dL)	89.10 \pm 9.93	85.29 \pm 9.11	89.18 \pm 8.52	93.05 \pm 10.60	<0.001
HDL-C (mg/dL)	55.70 \pm 14.05	60.93 \pm 14.85	55.59 \pm 13.17	50.28 \pm 11.94	<0.001
Insulin (μ U/mL)	7.10 \pm 4.14	3.60 \pm 0.94	6.21 \pm 0.86	11.72 \pm 4.02	<0.001
LDL-C (mg/dL)	121.48 \pm 32.05	118.90 \pm 34.55	126.03 \pm 31.01	119.53 \pm 30.10	0.20
T-cholesterol (mg/dL)	200.61 \pm 35.20	198.85 \pm 36.98	203.81 \pm 35.12	119.18 \pm 33.43	0.52
TG (mg/dL)	117.34 \pm 60.61	95.39 \pm 45.13	111.04 \pm 49.83	147.05 \pm 72.48	<0.001
Current smoking, n(%)	34 (10.6)	14 (12.7)	11 (10.3)	9 (8.7)	0.62
Marital status (single), n(%)	54 (16.8)	22 (20.0)	14 (13.1)	18 (17.3)	0.39
Men, n(%)	111 (34.6)	41 (37.3)	39 (36.4)	31 (29.8)	0.46
Regular exercise, n(%)	264 (82.2)	92 (83.6)	96 (89.7)	76 (73.1)	0.01
Vegetarian, n(%)	20 (6.2)	7 (6.4)	7 (6.5)	6 (5.8)	0.97
HTN, n(%)	150 (46.7)	43 (39.1)	47 (43.9)	60 (57.7)	0.02
Hyperlipidemia, n(%)	204 (63.6)	58 (52.7)	69 (64.5)	77 (74.0)	0.005
Metabolic syndrome, n(%)	90 (28.0)	11 (10.0)	23 (21.5)	56 (53.8)	<0.001

Notes: Clinical characteristics are expressed as the mean \pm SD for continuous variables and n(%) for categorical variables. P-values were derived from one-way analysis of variance (ANOVA) for continuous variables and chi-square test for categorical variables.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood

pressure; ALT, alanine aminotransferase; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HTN, hypertension.

When designating the low insulin level group as the reference, the middle and high insulin level groups had an OR of 2.46 ($p=0.02$) and 10.50 ($p<0.001$) for predicting MetS, respectively. After adjusting age, gender, and BMI, the middle and high insulin level groups had an OR of 1.71 ($p=0.20$) and 5.63 ($p<0.001$) for predicting MetS, respectively. After adjusting age, gender, BMI, smoking, hypertension and dyslipidemia, the middle and high insulin level groups still had an OR of 1.51 ($p=0.35$) and 5.04 ($p<0.001$; Table 4) for predicting MetS, respectively. There was no significant difference between the middle and low tertile groups, but a significant difference between the high and low tertile groups, even after adjusting for the above risk factors. Based on this data, the high insulin level group had a 5-fold times risk for MetS compared to the low insulin level group.

Figure 2 shows the ROC curve of the insulin level as a predictor for MetS. The AUC was 0.78. The optimized cut-off value for insulin was shown to be 7.35 $\mu\text{U/mL}$, with a sensitivity of 0.69 and a specificity of 0.77 (Table 5).

DISCUSSION

In this community-based study, we investigated fasting serum insulin levels in association with the prevalence of MetS in non-diabetic middle-aged and elderly Taiwanese adults. In our study, the prevalence of MetS in the relatively healthy middle age-to-elderly population was 28%, which is similar to the 29.75% findings reported by Li et al.²³ among middle-aged and elderly populations in Taiwan. Looking at the three groups as a function of fasting insulin tertiles, there was a rising proportion of MetS as the fasting insulin level increased. This finding not only applied to MetS, but to MetS-DCs as well. The WC, SBP, TG, and FPG levels were the lowest in the low fasting insulin group and highest in the high fasting insulin group; the converse applied to HDL and vice versa (Table 1). This finding led us speculate that an association exists between fasting insulin levels and MetS-DCs. We found a statistically significant correlation between fasting insulin levels and each MetS-DC, even after adjusting for age (Table 2). A trend existed between the fasting insulin level and the prevalence of all five MetS-DCs (Table 3). We thus wanted to know if the trend applied to the prevalence of MetS. Figure 1 shows an apparent increase in the prevalence of MetS as the fasting insulin level increased. The trend was confirmed by the Cochran-Armitage trend test ($p < 0.0001$).

Table 2 Pearson's correlation coefficients for each component of metabolic syndrome and age in relation to insulin levels.

Variables	Insulin(n=321)			
	Unadjusted		Adjusted for age	
	Pearson's	p value	Pearson's	p value
	coefficient		coefficient	
Age (year)	-0.04	0.50	NA	NA
SBP (mmHg)	0.21	<0.001	0.22	<0.001
DBP (mmHg)	0.11	0.05	0.10	0.07
Waist circumference (cm)	0.43	<0.001	0.44	<0.001
FPG (mg/dL)	0.38	<0.001	0.39	<0.001
HDL-C (mg/dL)	-0.37	<0.001	-0.37	<0.001
TG (mg/dL)	0.37	<0.001	0.37	<0.001

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

Table 3 Prevalence of components of metabolic syndrome based on insulin levels.

Components	Low	Middle	High	p value for Cochran- Armitage trend test
	(n=110) N(%)	(n=107) N(%)	(n=104) N(%)	
High blood pressure ^a	56(50.9)	63(58.9)	78(75)	0.0003
High blood glucose ^b	8(7.3)	10(9.3)	25(24.0)	0.0004
Low HDL-C ^c	15(13.6)	19(17.8)	43(41.3)	<0.0001
High TG ^d	17(15.5)	27(25.2)	42(40.4)	<0.0001
Central obesity ^e	34(30.9)	57(53.3)	82(78.8)	<0.0001

Note:

^a SBP \geq 130 mmHg or DBP \geq 85 mmHg, or self-reported hypertension

^b Fasting blood glucose \geq 100 mg/dL or self-reported diabetes mellitus

^c HDL-C < 40 mg/dL in men or < 50 mg/dL in women

^d TG \geq 150 mg/dL

^e Waist circumference \geq 90 cm in men or \geq 80 cm in women

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

After adjusting for gender, age, BMI, smoking status, hypertension, and dyslipidemia, the middle-aged and elderly populations in the high fasting insulin group were at significant risk for developing MetS (OR = 5.04, 95% CI =2.15-11.81; $P < 0.01$; Table 4). This conclusion is consistent with the finding of Sung et al.¹⁹, who reported that higher fasting insulin levels increased the risk of acquiring MetS. One possible explanation might be the relationship between the fasting insulin level and insulin resistance, which has a fundamental role in MetS^{24 25}. Although the mechanism by which fasting insulin may induce insulin resistance was not investigated in the present study, a number of studies have shown that fasting insulin is a suitable surrogate marker for insulin resistance²⁶⁻²⁹, calculated by the fasting insulin resistance index (FIRI) or homeostasis model assessment of insulin resistance (HOMA-IR). Based on these equations, a higher fasting insulin level can contribute to insulin resistance in patients with impaired fasting glucose, but may be an inappropriate marker in diabetics with poor glycemic control. This finding may be the reason that a higher fasting insulin level increases the risk of acquiring MetS in non-diabetic populations. It has been reported that the fasting insulin level is highly associated with MetS, but a cutoff value was not established²¹. Based on our search of the literature, there is no widely accepted reference range for fasting insulin. A reference range for fasting insulin of 1.57–16.32 $\mu\text{U/mL}$ has been proposed in Chinese men, but the reference range varies between different ethnicities and genders³⁰. A fasting insulin level $> 9\mu\text{U/mL}$ has been reported to identify 80% of patients with pre-diabetes³¹. In our study, the AUC for fasting insulin as a predictor for MetS was 0.78, and our proposed cut-off value for fasting insulin was 7.35 $\mu\text{U/mL}$ (sensitivity=0.69 and specificity=0.77; Table 5). Because MetS is a state of pre-diabetes²⁵, our cutoff value $< 9\mu\text{U/mL}$ is reasonable. Our proposed cut-off value of 7.35 $\mu\text{U/mL}$ is between the mean fasting insulin levels of the middle and high tertile groups. This may be reasonable because the middle and low tertile groups did not show a statistically significant difference in OR for

MetS after adjusting for confounding factors.

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Table 4 Association between insulin levels and metabolic syndrome.

Variables	Model 1			Model 2			Model 3		
	OR	(95%CI)	p value	OR	(95%CI)	p value	OR	(95%CI)	p value
Low	1.00	—	—	1.00	—	—	1.00	—	—
Middle	2.46	(1.14-5.35)	0.02	1.71	(0.76-3.85)	0.20	1.51	(0.64-3.57)	0.35
High	10.50	(5.05-21.84)	<0.001	5.63	(2.53-12.53)	<0.001	5.04	(2.15-11.81)	<0.001
p value for trend			<0.001			<0.001			<0.001

Model 1: unadjusted

Model 2: adjusted for gender, age, and BMI

Model 3: adjusted for factors in model 2 plus smoking, HTN, and hyperlipidemia

Abbreviations: BMI, body mass index; HTN, hypertension; OR, odds ratio; CI, confidence interval.

Table 5 The areas under the ROC curve (AUC), sensitivity, and specificity at the optimized cut-off point for insulin in predicting metabolic syndrome.

Variables	AUC(95% CI)	p value	Cut-off point	Sensitivity	Specificity
Insulin (μU/mL)	0.78(0.72-0.84)	<0.001	7.35	0.69	0.77

Abbreviations: ROC curve, receiver operating characteristic curve; CI, confidence interval.

Our findings may have an impact on health screening policies in people older than middle-age. Aggressive lifestyle modification should be promptly instituted when the fasting insulin level is $> 7.35 \mu\text{U/mL}$, and in the case of progression, some medical interventions should be considered.

Some limitations in our study merit consideration. First, the principal limitation relevant to the interpretation of our results was the use of a cross-sectional design, thus a causal relationship between the fasting insulin level and MetS cannot be inferred. Second, the sample size in our study was relatively small ($n=321$ [the power was not calculated]) and the participants were recruited from a regional community. The participants could therefore only be distributed into three groups and the results cannot be generalized to other ethnicities. Furthermore, even though we used a standardized questionnaire, recall and reporting bias are unavoidable for self-reported data. Finally, we did not ask participants to sleep adequately or to avoid vigorous exercise the day before blood testing, which could affect the accuracy of the fasting serum insulin level.

Our study also has strengths. First, our participants were recruited during a community health examination and represent a relatively healthy population. The effects of important confounders, including ethnicity, residential area, and environmental factors, were minimized. Second, we used standardized laboratory examination protocols and anthropometric measurements. Third, while evaluating the association between MetS and the fasting insulin level, we excluded diabetic patients to avoid the effect of anti-diabetic medications on the fasting insulin level.

In the future, we will continue to follow this community and record the development of newly diagnosed MetS.

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CONCLUSIONS

This study provides another convenient method to predict the existence of subsequent MetS by testing fasting insulin levels in the in the middle-aged and elderly non-diabetic populations. We suggest a cut-off value of 7.35 μ U/mL for MetS. We believe that the cut-off value is a robust and reliable predictor which facilitates the early detection of MetS. Providing medical counseling to patients with a fasting insulin level $> 7.35 \mu$ U/mL should result in long-term health benefits.

Contributors YHC and YCL were involved in writing of the manuscript. YCT, MCL, HHC and WCY conceived and supervised the study. IST provided statistical advice. JYC contributed conceived, designed and performed the experiments, collected and analyzed the data, revising it critically for important intellectual content and final approval of the version to be submitted.

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Competing interests None declared.

Ethics approval The study was approved by Chang-Gung Medical Foundation Institutional Review Board (102-2304B), and written informed consent was given by all the participants before enrollment.

Data sharing statement No additional data are available.

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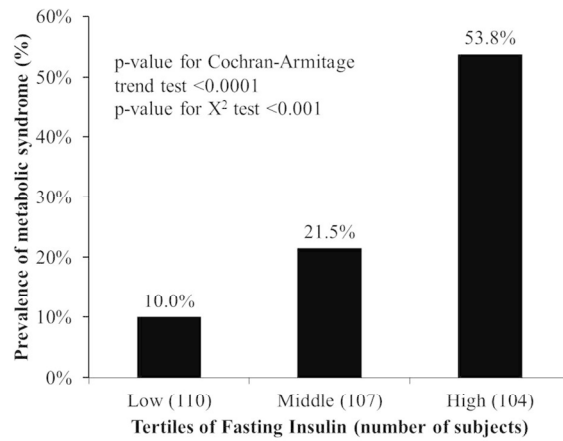


Figure 1 Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

Figure 1 Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

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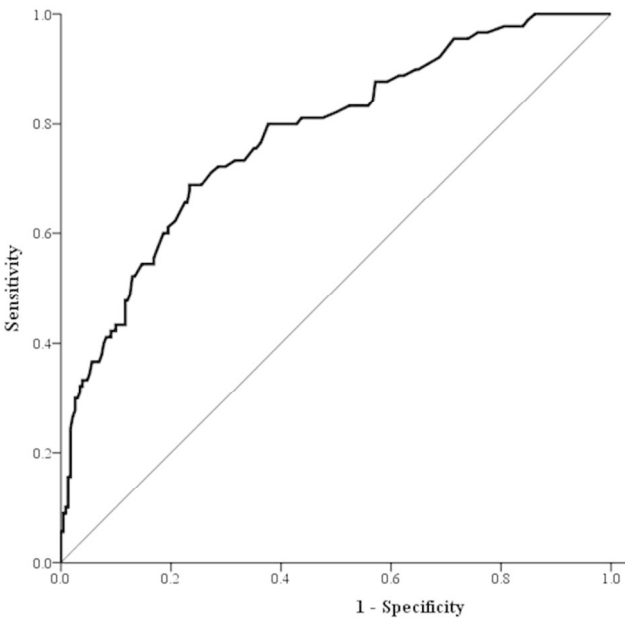


Figure 2 ROC curve for insulin as a predictor of metabolic syndrome.

Figure 2 ROC curve for insulin as a predictor of metabolic syndrome.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	P2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	P2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any prespecified hypotheses	P2, P4
Methods			
Study design	4	Present key elements of study design early in the paper	P4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P4, 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	P4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	Nil
Study size	10	Explain how the study size was arrived at	Nil
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	P6
		(b) Describe any methods used to examine subgroups and interactions	P6
		(c) Explain how missing data were addressed	Nil
		(d) If applicable, describe analytical methods taking account of sampling strategy	Nil
		(e) Describe any sensitivity analyses	Nil
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Nil
		(b) Give reasons for non-participation at each stage	Nil
		(c) Consider use of a flow diagram	Nil
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P8
		(b) Indicate number of participants with missing data for each variable of interest	Nil
Outcome data	15*	Report numbers of outcome events or summary measures	P6-9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P7
		(b) Report category boundaries when continuous variables were categorized	P8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Nil
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Nil
Discussion			
Key results	18	Summarise key results with reference to study objectives	P10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P13
Generalisability	21	Discuss the generalisability (external validity) of the study results	P16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

High fasting insulin levels are associated with metabolic syndrome in non-diabetic middle-aged and elderly populations: A community-based study in Taiwan

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Keywords:	metabolic syndrome, insulin resistance, fasting insulin level

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1 ● **Title**

2 High fasting insulin levels are associated with metabolic syndrome in non-diabetic middle-
3 aged and elderly populations: A community-based study in Taiwan

4 ● **Short title**

5 Increased fasting insulin levels in association with metabolic syndrome

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ABSTRACT

Objectives: We determined the association on between fasting insulin (FI) levels and metabolic syndrome (MetS), and suggested a value of (FI) which can help detect possible comorbidity of MetS in non-diabetic middle-aged and elder adults.

Design: Cross-sectional observational study.

Setting: Community-based investigation in Guishantownship of northern Taiwan.

Participants: Our study included adults aged 50 years and above during community health exams between January and October 2014. People with diabetes mellitus were excluded. A total of 321 people were enrolled.

Outcome measures: We divided participants according to tertiles of FI as low, medium, and high levels. Pearson correlation was assessed between insulin level and each of the diagnostic components of metabolic syndrome (MetS-DCs) with adjustment of age. The prevalence of MetS-DCs based on tertiles of FI were studied and analyzed by Cochran-Armitage trend test. The risk for prevalence of MetS in the middle, and high insulin group as compared to the low insulin group were assessed by multivariate logistic regression with adjustments for age, gender, smoking, body mass index, hypertension, and hyperlipidemia. Youden Index was performed for the optimized cut-off value.

Results: Our results showed positive correlation of FI level with systolic blood pressure, waist circumference, fasting plasma glucose and triglyceride levels, while negative correlation was shown with high-density lipoprotein ($p < 0.001$). The prevalence of each MetS-DCs increased as a trend while FI levels increased ($p < 0.001$). OR (95% CI) of MetS was 5.04(2.15-11.81) for high insulin groups as compared to the low insulin group after adjusting confounders ($p < 0.001$). Area under ROC curve (AUC) was 0.78, and cut-off value 7.35 μ U/mL for FI was obtained (sensitivity: 0.69; specificity: 0.77).

Conclusions: People with increased FI are associated with a higher prevalence of MetS. The

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proposed cut-off value of FI may act as a marker for increased risk of MetS in middle-aged and elderly non-diabetic adults.

Keywords: metabolic syndrome, insulin resistance, fasting insulin level

Strengths and limitations of this study:

- This is the first study to explore the relationship between fasting insulin and MetS in the middle-aged and elderly populations of non-diabetic Asian people.
- We offer an easily measured biomarker to identify Chinese middle-aged and elderly with MetS.
- We adjusted many confounding factors to make the results more reliable.
- The recall bias from self-reported lifestyle behaviors is unavoidable.
- The causal relationship between fasting insulin level and MetS cannot be demonstrated in our cross-sectional study.

INTRODUCTION

Metabolic syndrome (MetS) is associated with a cluster of unhealthy metabolic risk factors, including abdominal obesity (excess body fat around the waist), glucose intolerance, pre-morbid hypertension, and dyslipidemia¹⁻³. A number of studies have reported that MetS increases the risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and other non-communicable diseases (NCDs)⁴⁻⁷. The rising prevalence of MetS has created a disturbing challenge to personal health⁸⁻¹².

Insulin resistance has long been associated with MetS¹³⁻¹⁶. Basal insulin represents 45%-50% of daily insulin^{17 18}. And the FI level approximates basal insulin^{18 19}. Studies have shown that FI levels may be associated with the prevalence of MetS, which may be due to its representativeness of insulin resistance^{20 21}. A study has even shown that elevated FI levels may predict the future incidence of MetS¹⁹. According to DeBoer et al. different ethnics may reach the criteria for MetS at different stages of insulin resistance²². If insulin resistance is the foundation of MetS^{14 15}, and FI represents insulin resistance with an area under curve(AUC)[95% confidence interval(CI)] of 0.995[0.993-0.996]²⁰, a high FI level may be able to caution the physician for susceptibility to metabolic diseases and hence cardiovascular risks²³. We therefore determined the relationships between fasting plasma insulin and MetS in middle-aged and elderly populations. In addition, we also determined a cut-off value for FI as a biomarker for the prevalence of MetS. By determining a cutoff value for FI, we could educate patients at risk for MetS and recommend lifestyle modification at an earlier stage.

METHODS

Study subjects

This was an observational and cross-sectional study conducted at Linkou Chang Gung Memorial Hospital in Taoyuan County, Taiwan between January and October 2014. The

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90 inclusion criteria included residents 50-90 years old and living in Guishan township. 619
91 residents were eligible for the study. A total of 400 residents agreed to participate in our
92 health exam. Subjects were excluded if they had diabetes. 79 participants with diabetes
93 mellitus were excluded. A total of 321 participants (111 males and 210 females) were
94 ultimately enrolled for analysis. This study was approved by the Institutional Review Board
95 of the hospital and written inform consent was obtained from all of the participants before
96 enrollment.

97 **Data collection**

98 We obtained exercise (exercising ≥ 3 times a week or not) and dietary habits (vegetarian
99 or not) from self-administered questionnaires, which also included smoking (current smoker
100 or not) and marital status (currently married or not). Anthropometric data, such as height,
101 weight, waist circumference (WC), and blood pressure were also recorded. The subjects were
102 dressed in light clothing without shoes for weight and height measurements. The BMI was
103 calculated as the weight in kilograms (kg) divided by the height in meters squared (m^2). Waist
104 circumference was measured midway between the inferior margin of the lowest rib and the
105 iliac crest in the horizontal plane while in an upright position. Systolic blood pressure (SBP)
106 and diastolic blood pressure (DBP) were checked at least 2 times after 5 min of rest while
107 seated. Fasting insulin levels, lipid profile, and fasting glucose were obtained by blood
108 sampling after a 10-h overnight fast. Blood samples were analyzed in the central laboratory of
109 Linkou Chang Gung Memorial Hospital for fasting plasma glucose (FPG), serum total
110 cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-
111 cholesterol (HDL-C), serum triglycerides (TG), and fasting insulin levels. Serum insulin
112 levels were determined with an ARCHITECT Insulin assay (Abbott Laboratories, IL, USA).
113 Insulin was measured with a chemiluminescent microparticle immunoassay (CMIA). The intra-
114 assay variation and inter-assay variations were less than 2.7%. The ARCHITECT Insulin

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3 115 assay has a sensitivity of $\leq 1.0 \mu\text{U/ml}$.
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5 116 **Defining MetS**

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7 117 MetS was defined by at least 3 of 5 metabolic syndrome diagnostic components (MetS-
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9 118 DCs), according to The Third Report of the National Cholesterol Education Program Expert
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11 119 Panel (NCEP) on Adult Treatment Panel (ATP III) Asian diagnostic criteria²⁴. The five MetS-
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13 120 DCs were as follows: 1) SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg, or the use of anti-
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15 121 hypertensive drugs; 2) decreased serum HDL-C concentration < 40 mg/dl in men and < 50
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17 122 mg/dL in women, or treatment for dyslipidemia; 3) TG concentration ≥ 150 mg/dl, or on
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19 123 medication for hypertriglyceridemia; 4) hyperglycemia: fasting plasma glucose level ≥ 100
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21 124 mg/dl; and 5) abdominal WC ≥ 90 cm in men or ≥ 80 cm in women.
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25 125 **Statistical Analysis**

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27 126 Subjects were classified in one of three groups according to serum insulin level tertiles as
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29 127 the low, middle, and high insulin groups. Clinical characteristics were expressed as the mean
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31 128 \pm standard deviation (SD) for continuous variables and number (%) for categorical variables.
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33 129 One-way analysis of variance (ANOVA) or a Chi-square test was used to determine p-values
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35 130 for continuous or categorical variables, respectively. Pearson's correlation was performed for
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37 131 each MetS-DC in relation to fasting insulin levels. The Cochran-Armitage trend test was used
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39 132 to evaluate the increasing prevalence of MetS-DCs as a function of insulin level tertile. The
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41 133 low fasting insulin group was designated as the reference group to calculate the ORs of the
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43 134 prevalence of MetS in the middle and high fasting insulin groups using multivariate logistic
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45 135 regression. Confounded variables present as an obstacle to valid inference in MetS studies.
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47 136 Hypertension and dyslipidemia are both common chronic conditions that affect a large
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49 137 proportion of the general adult population. Previous studies determining the association of FI
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51 138 and MetS also adjusted MetS-DCs¹⁹. Results of the adjusted model provide valid inference
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53 139 among MetS and insulin levels. A receiver operating characteristic curve (ROC) curve was
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created for fasting insulin as a predictor of MetS. The area under the ROC curve (AUC) was analyzed, and the optimized cut-off point for fasting insulin, sensitivity, and specificity were acquired using the maximal Youden’s Index. We used SPSS (version 23.0 for windows), to perform the statistical analysis. Statistical significance was set at a p-value < 0.05.

RESULTS

A total of 321 individuals, 111 men (34.6%) and 210 (65.4%) women, with a mean age of 63.91±8.32 years, were enrolled in this study. There were 90 study participants (28%) who met the diagnosis of MetS.

Table 1 shows the characteristics of the study population, which was divided based on the fasting insulin level in µU/mL. There were no statistically significant differences in age or gender between the low, middle, and high insulin level groups, while differences did exist with respect to WC, SBP, HDL-C, TG, and the proportion with MetS. Table 2 further shows the correlation between the fasting insulin level and all MetS-DCs, even after adjusting for age. Fasting insulin was positively correlated with SBP, WC, FPG, and TG, and negatively correlated with HDL-C, as shown in Table 2. Table 3 shows the prevalence of MetS-DCs (hypertension, hyperglycemia, dyslipidemia, and central obesity) according to the insulin level tertiles. The prevalence of MetS-DCs increased as the fasting insulin level increased, as shown by significant p values (Cochran-Armitage trend test). Figure 1 shows that the low insulin level group had a 10% prevalence of MetS, the middle insulin level group had a 21.5% prevalence of MetS, and the high insulin level group had a 53.8% of MetS (p<0.0001 [Cochran-Armitage trend test]), suggesting that the prevalence of MetS increased with an increase in fasting insulin levels.

Table 1 General characteristics of the study population based on insulin levels.

Variables	Insulin levels				p value
	Total n=321	Low n=110 (≤ 4.8)	Middle n=107 (4.9-7.8)	High n=104 (≥ 7.9)	
Age (year)	63.91 \pm 8.32	64.23 \pm 8.32	64.47 \pm 8.67	63.01 \pm 7.93	0.40
BMI (kg/m ²)	24.36 \pm 3.53	22.41 \pm 3.14	24.41 \pm 2.73	26.37 \pm 3.54	<0.001
Waist circumference (cm)	84.23 \pm 9.51	79.69 \pm 7.57	83.78 \pm 8.63	89.51 \pm 9.65	<0.001
SBP (mmHg)	129.02 \pm 16.59	123.69 \pm 17.36	129.52 \pm 14.51	134.13 \pm 16.20	<0.001
DBP (mmHg)	77.01 \pm 10.90	75.43 \pm 11.80	76.92 \pm 10.03	78.79 \pm 10.60	0.08
ALT (U/L)	21.74 \pm 11.06	18.94 \pm 7.81	20.33 \pm 9.25	26.15 \pm 14.05	<0.001
Creatinine (mg/dL)	0.76 \pm 0.44	0.69 \pm 0.17	0.85 \pm 0.66	0.75 \pm 0.34	0.03
FPG (mg/dL)	89.10 \pm 9.93	85.29 \pm 9.11	89.18 \pm 8.52	93.05 \pm 10.60	<0.001
HDL-C (mg/dL)	55.70 \pm 14.05	60.93 \pm 14.85	55.59 \pm 13.17	50.28 \pm 11.94	<0.001
Insulin (μ U/mL)	7.10 \pm 4.14	3.60 \pm 0.94	6.21 \pm 0.86	11.72 \pm 4.02	<0.001
LDL-C (mg/dL)	121.48 \pm 32.05	118.90 \pm 34.55	126.03 \pm 31.01	119.53 \pm 30.10	0.20
T-cholesterol (mg/dL)	200.61 \pm 35.20	198.85 \pm 36.98	203.81 \pm 35.12	119.18 \pm 33.43	0.52
TG (mg/dL)	117.34 \pm 60.61	95.39 \pm 45.13	111.04 \pm 49.83	147.05 \pm 72.48	<0.001
Current smoking, n(%)	34 (10.6)	14 (12.7)	11 (10.3)	9 (8.7)	0.62
Marital status (single), n(%)	54 (16.8)	22 (20.0)	14 (13.1)	18 (17.3)	0.39
Men, n(%)	111 (34.6)	41 (37.3)	39 (36.4)	31 (29.8)	0.46
Regular exercise, n(%)	264 (82.2)	92 (83.6)	96 (89.7)	76 (73.1)	0.01
Vegetarian, n(%)	20 (6.2)	7 (6.4)	7 (6.5)	6 (5.8)	0.97
HTN, n(%)	150 (46.7)	43 (39.1)	47 (43.9)	60 (57.7)	0.02
Hyperlipidemia, n(%)	204 (63.6)	58 (52.7)	69 (64.5)	77 (74.0)	0.005
Metabolic syndrome, n(%)	90 (28.0)	11 (10.0)	23 (21.5)	56 (53.8)	<0.001

Notes: Ranges of FI levels of different tertile groups are shown in brackets at the top of the table, units in μ U/mL.

Clinical characteristics are expressed as the mean \pm SD for continuous variables and n(%) for categorical variables. P-values were derived from one-way analysis of variance (ANOVA) for

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continuous variables and chi-square test for categorical variables.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HTN, hypertension.

When designating the low insulin level group as the reference, the middle and high insulin level groups had an OR of 2.46 ($p=0.02$) and 10.50 ($p<0.001$) for MetS, respectively. After adjusting age, gender, and BMI, the middle and high insulin level groups had an OR of 1.71 ($p= 0.20$) and 5.63 ($p <0.001$) for MetS, respectively. After adjusting age, gender, BMI, smoking, hypertension and dyslipidemia, the middle and high insulin level groups still had an OR of 1.51 ($p=0.35$) and 5.04 ($p<0.001$; Table 4) for MetS, respectively. There was no significant difference between the middle and low tertile groups, but a significant difference between the high and low tertile groups, even after adjusting for the above risk factors. Based on this data, the high insulin level group had a 5-fold times risk for MetS compared to the low insulin level group.

Figure 2 shows the ROC curve of the insulin level as a predictor for MetS. The AUC was 0.78. The optimized cut-off value for insulin was shown to be 7.35 $\mu\text{U/mL}$, with a sensitivity of 0.69 and a specificity of 0.77.

DISCUSSION

In this community-based study, we investigated fasting serum insulin levels in association with the prevalence of MetS in non-diabetic middle-aged and elderly Taiwanese adults. In our study, the prevalence of MetS in the relatively healthy middle age-to-elderly population was 28%, which is similar to the 29.75% findings reported by Li et al.²⁵ Among middle-aged and elderly populations in Taiwan. Looking at the three groups as a function of fasting insulin tertiles, there was a rising proportion of MetS as the fasting insulin level increased, also shown in previous studies^{20 26 27}. This finding not only applied to MetS, but to MetS-DCs as well. The WC, SBP, TG, and FPG levels were the lowest in the low fasting insulin group and highest in the high fasting insulin group; the converse applied to HDL and vice versa (Table 1). This finding led us to speculate that an association exists between fasting insulin levels and MetS-DCs. We found a statistically significant correlation between fasting insulin levels and each MetS-DC, even after adjusting for age (Table 2), in accordance to a study in adolescents²². A trend existed between the fasting insulin level and the prevalence of all five MetS-DCs (Table 3). We thus wanted to know if the trend applied to the prevalence of MetS. Figure 1 shows an apparent increase in the prevalence of MetS as the fasting insulin level increased. The trend was confirmed by the Cochran-Armitage trend test ($p < 0.0001$).

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Table 2 Pearson's correlation coefficients for each component of metabolic syndrome and age in relation to insulin levels.

Variables	Insulin(n=321)			
	Unadjusted		Adjusted for age	
	Pearson's	p value	Pearson's	p value
	coefficient		coefficient	
Age (year)	-0.04	0.50	NA	NA
SBP (mmHg)	0.21	<0.001	0.22	<0.001
DBP (mmHg)	0.11	0.05	0.10	0.07
Waist circumference (cm)	0.43	<0.001	0.44	<0.001
FPG (mg/dL)	0.38	<0.001	0.39	<0.001
HDL-C (mg/dL)	-0.37	<0.001	-0.37	<0.001
TG (mg/dL)	0.37	<0.001	0.37	<0.001

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

Table 3 Prevalence of components of metabolic syndrome based on insulin levels.

Components	Low (n=110) N(%)	Middle (n=107) N(%)	High (n=104) N(%)	p value for Cochran- Armitage trend test
High blood pressure ^a	56(50.9)	63(58.9)	78(75)	0.0003
High blood glucose ^b	8(7.3)	10(9.3)	25(24.0)	0.0004
Low HDL-C ^c	15(13.6)	19(17.8)	43(41.3)	<0.0001
High TG ^d	17(15.5)	27(25.2)	42(40.4)	<0.0001
Central obesity ^e	34(30.9)	57(53.3)	82(78.8)	<0.0001

Note:

^a SBP \geq 130 mmHg or DBP \geq 85 mmHg, or self-reported hypertension

^b Fasting blood glucose \geq 100 mg/dL or self-reported diabetes mellitus

^c HDL-C < 40 mg/dL in men or < 50 mg/dL in women

^d TG \geq 150 mg/dL

^e Waist circumference \geq 90 cm in men or \geq 80 cm in women

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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215 After adjusting for gender, age, BMI, smoking status, hypertension, and dyslipidemia,
216 the middle-aged and elderly populations in the high fasting insulin group were at significant
217 risk for developing MetS (OR = 5.04, 95% CI =2.15-11.81; P<0.01: Table 4). This
218 conclusion is consistent with previous findings^{20 27 28}. Not only is fasting insulin an
219 independent risk factor for MetS, but in the cohort study of Sung et al., it was also reported
220 that elevated fasting insulin predicted the future incidence of MetS¹⁹. One possible
221 explanation might be the relationship between the fasting insulin level and insulin resistance²⁰
222 ²¹, which has a fundamental role in MetS^{29 30}. Although the mechanism by which fasting
223 insulin may represent insulin resistance was not investigated in the present study, a number of
224 studies have shown that fasting insulin is a suitable surrogate marker for insulin resistance²¹
225 ³¹⁻³⁴, calculated by the fasting insulin resistance index (FIRI) or homeostasis model
226 assessment of insulin resistance (HOMA-IR). Based on these equations, a higher fasting
227 insulin level can contribute to insulin resistance in patients with impaired fasting glucose, but
228 may be an inappropriate marker in diabetics with poor glycemic control. This finding may be
229 the reason that a higher fasting insulin level increases the risk of MetS in non-diabetic
230 populations. It has been reported that the fasting insulin level is highly associated with MetS,
231 but a cutoff value was not established ²⁰. Based on our search of the literature, there is no
232 widely accepted reference range for fasting insulin. A reference range for fasting insulin of
233 1.57–16.32 µU/mL has been proposed in Chinese men, but the reference range varies between
234 different ethnicities and genders ³⁵. A fasting insulin level > 9µU/mL has been reported to
235 identify 80% of patients with pre-diabetes ³⁶. In our study, the AUC for fasting insulin as an
236 indication of risk for MetS was 0.78, similar to another study’s AUC of 0.77²⁰. Our proposed
237 cut-off value for fasting insulin was 7.35 µU/mL (sensitivity=0.69 and specificity=0.77).
238 Because MetS is a state of pre-diabetes³⁰, our cutoff value < 9µU/mL is reasonable. Our
239 proposed cut-off value of 7.35 µU/mL is between the mean fasting insulin levels of the

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3 240 middle and high tertile groups. This may be reasonable because the middle and low tertile
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5 241 groups did not show a statistically significant difference in OR for MetS after adjusting for
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7 242 confounding factors.
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Table 4 Association between insulin levels and metabolic syndrome.

Variables	Model 1			Model 2			Model 3		
	OR	(95% CI)	p value	OR	(95% CI)	p value	OR	(95% CI)	p value
Low	1.00	—	—	1.00	—	—	1.00	—	—
Middle	2.46	(1.14-5.35)	0.02	1.71	(0.76-3.85)	0.20	1.51	(0.64-3.57)	0.35
High	10.50	(5.05-21.84)	<0.001	5.63	(2.53-12.53)	<0.001	5.04	(2.15-11.81)	<0.001
p value for trend			<0.001			<0.001			<0.001

Model 1: unadjusted

Model 2: adjusted for gender, age, and BMI

Model 3: adjusted for factors in model 2 plus smoking, HTN, and hyperlipidemia

Abbreviations: BMI, body mass index; HTN, hypertension; OR, odds ratio; CI, confidence interval.

Table 5 Studies of Association Between Fasting Insulin and Metabolic Syndrome.

Authors	Study Year	Study Population	% MetS	Fasting insulin	Risk of MetS	Main Finding	Reference
Saravia G et al 2015	2009-2010 Cross sectional	3200 Non-diabetic males in Spain	23%	Highest tertile (≥ 6.13) vs lowest (≤ 3.80) $\mu\text{U/mL}$	OR (95%CI) 11.36 (8.65-15.13) for MetS	Per each 10 pmol/L (1.4 uU/mL) increase in insulin, the odds for metabolic syndrome increased by 1.43 (95%CI: 1.38, 1.49)	[20]
Rutter MK et al 2014	(1991-1995) to (1998-2001) 7 year Prospective	2616 non-diabetic adults in Europe	-	1-quintile change in fasting insulin (pmol/L)	mean (95% CI) 0.12 (0.10–0.15) [MetS trait score 7 year change]	Change in metabolic trait clustering was significantly associated with baseline levels and changes in fasting insulin.	[38]
Sung KC et al 2011	2003-2008 5 year cohort	2350 non-MetS in Korea	8.5% (incidence)	Highest quartile (≥ 8.98) vs lowest (≤ 6.01) IU/ml	OR (95% CI) of developing MS 5.1 (3.1-8.2)	The highest quartile of the insulin levels had more than a 5 times greater risk of developing MS compared to the subjects in the lowest quartile.	[19]
Kanda H et al 2011	2000,2001 Cross sectional	456 in Mongolia	6.4%	Highest tertile (≥ 10.33) vs lowest (≤ 6.72) mmol/L	Percentage of MetS 17.1% vs 4.6%	Fasting plasma insulin is associated with MetS in farmers, but not nomads among the Mongolian population in China.	[27]
STOPP-T2D PSG* 2008	2003 Cross sectional	1453 8 th grade adolescents in the U.S.	9.5%	Highest quintile (≥ 39.1) vs lowest (≤ 17.0) $\mu\text{U/mL}$	OR (95%CI) 199.64(31.29-1273.7) for MetS	The highest insulin quintile were almost 200 times more likely to be classified with the metabolic syndrome than participants in the lowest quintile.	[28]
Adam FM et al 2006	2005 Cross sectional	128 overweight/obese in Indonesia	68.8%	Mean fasting insulin levels 15.68 ± 7.85 vs 3.16 ± 2.53 (uU/ml) with 5 components vs 1 component of MetS.		There is a strong linear increase in fasting insulin levels with an increase of the number of metabolic syndrome.	[29]

*STOPP-T2D PSG: Studies to Treat or Prevent Pediatric Type 2 Diabetes Prevention Study Group

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Our findings may have an impact on health screening policies in people older than middle-age. The diagnosis criteria of MetS differs according to gender, ethnicity and even age. A study shows that certain ethnic groups do not meet current criteria of MetS until they have reached a more advanced degree of insulin resistance²². Elevated FI, however, may act as a marker to alert physicians on the risk of MetS in this individual. Given the fact that elevated FI is not only associated with a greater risk for developing MetS^{19 37} but is also associated with a greater number of cardiometabolic risk factors²³, lifestyle modification should be considered when the FI level is > 7.35 µU/mL, due to the amelioration of metabolic abnormalities with diet or exercise interventions³⁸⁻⁴⁰.

Some limitations in our study merit consideration. First, the principal limitation relevant to the interpretation of our results was the use of a cross-sectional design, thus a causal relationship between the FI level and MetS cannot be inferred. Second, the sample size in our study was relatively small (n=321[the power was not calculated]) and the participants were recruited from a regional community. The participants could therefore only be distributed into three groups and the results cannot be generalized to other ethnicities. Third, though males tend to have a lower participation rate in studies⁴¹, there may still have been a selection bias due to the higher participation of women than men in our study. Fourth, according to our cutoff value, there is a false positive rate of 23% and a false negative rate of 31%. The false positive rate can be accepted due to our proposed intervention, early lifestyle modification, is less likely to do harm to the population. The false negative rate should be taken into consideration of the physician when applying this data. Furthermore, even though we used a standardized questionnaire, recall and reporting bias are unavoidable for self-reported data. Finally, we did not ask participants to sleep adequately or to avoid vigorous exercise the day before blood testing, which could affect the accuracy of the fasting serum insulin level.

Our study also has strengths. First, our participants were recruited during a community

health examination and represent a relatively healthy population. The effects of important confounders, including ethnicity, residential area, and environmental factors, were minimized. Second, we used standardized laboratory examination protocols and anthropometric measurements. Third, while evaluating the association between MetS and the fasting insulin level, we excluded diabetic patients to avoid the effect of anti-diabetic medications on the fasting insulin level. Lastly, due to the trend of world aging, our study aimed for middle-aged and elderly populations. And given the differences of FI levels in different ethnic groups²², our study contributes to the Taiwanese population. Studies from all around the world indicate the relationship of FI and MetS (table 5), though few physicians have applied to their practice.

In the future, we will continue to follow this community and record the development of newly diagnosed MetS. Counseling of lifestyle modification for residents with elevated FI will also be our topic of interest hereon.

CONCLUSIONS

This study provides a convenient method to identify the risk of MetS by testing FI levels in the non-diabetic populations. We suggest a FI cut-off value of 7.35 $\mu\text{U/mL}$ to start lifestyle modifications in the middle-aged and elderly non-diabetic population. We believe that the cut-off value can be of use to physicians, which cautions the risk of MetS. Providing medical counseling to patients with a FI level $>7.35 \mu\text{U/mL}$ should result in long-term health benefits. But further studies may be needed for this conclusion.

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Contributors YHC and YCL were involved in writing of the manuscript. YCT, MCL, HHC and WCY conceived and supervised the study. IST provided statistical advice. JYC contributed conceived, designed and performed the experiments, collected and analyzed the data, revising it critically for important intellectual content and final approval of the version to be submitted.

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Competing interests None declared.

Ethics approval The study was approved by Chang-Gung Medical Foundation Institutional Review Board (102-2304B), and written informed consent was given by all the participants before enrollment.

Data sharing statement No additional data are available.

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428 **Figure Legends**

429 **Figure 1.** Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend
430 across insulin tertiles.

431 **Figure 2.** ROC curve for insulin as a predictor of metabolic syndrome.

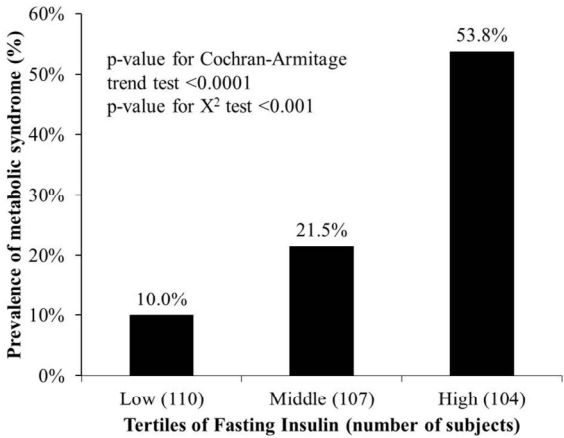


Figure 1 Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

Figure 1. Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

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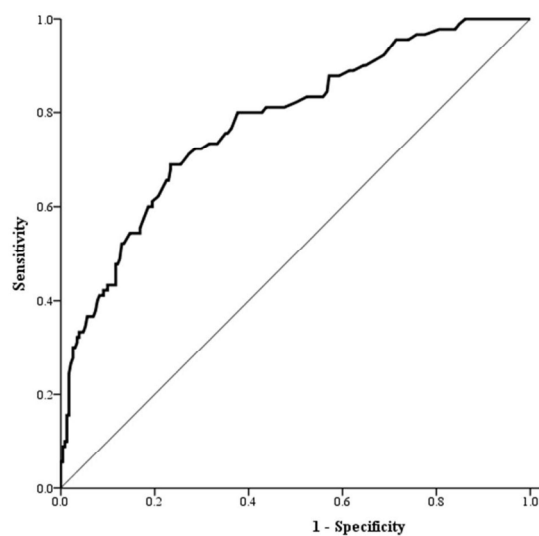


Figure 2 ROC curve for insulin as a predictor of metabolic syndrome.

Figure 2. ROC curve for insulin as a predictor of metabolic syndrome.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	P2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	P2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any prespecified hypotheses	P2, P4
Methods			
Study design	4	Present key elements of study design early in the paper	P4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P4, 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	P4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	Nil
Study size	10	Explain how the study size was arrived at	Nil
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	P6
		(b) Describe any methods used to examine subgroups and interactions	P6
		(c) Explain how missing data were addressed	Nil
		(d) If applicable, describe analytical methods taking account of sampling strategy	Nil
		(e) Describe any sensitivity analyses	Nil
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Nil
		(b) Give reasons for non-participation at each stage	Nil
		(c) Consider use of a flow diagram	Nil
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P8
		(b) Indicate number of participants with missing data for each variable of interest	Nil
Outcome data	15*	Report numbers of outcome events or summary measures	P6-9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P7
		(b) Report category boundaries when continuous variables were categorized	P8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Nil
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Nil
Discussion			
Key results	18	Summarise key results with reference to study objectives	P7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P17
Generalisability	21	Discuss the generalisability (external validity) of the study results	P18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

High fasting insulin levels are associated with metabolic syndrome in non-diabetic middle-aged and elderly populations: A community-based study in Taiwan

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Keywords:	metabolic syndrome, insulin resistance, fasting insulin level

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- 1 ● **Title**
- 2 High fasting insulin levels are associated with metabolic syndrome in non-diabetic middle-
- 3 aged and elderly populations: A community-based study in Taiwan
- 4 ● **Short title**
- 5 Increased fasting insulin levels in association with metabolic syndrome
- 6 ● **Author names and affiliations**
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- 21 ● **Format**
- 22 Word count: 2500
- 23 Number of black and white figures: 2
- 24 Number of Tables: 5
- 25 Number of References: 37
- 26

ABSTRACT

Objectives: We determined the association between fasting insulin (FI) levels and metabolic syndrome (MetS), and suggested a value of FI which can help detect possible comorbidity of MetS in non-diabetic middle-aged and elderly adults.

Design: Cross-sectional observational study.

Setting: Community-based investigation in Guishan township of northern Taiwan.

Participants: Our study included adults aged 50 years and above during community health exams between January and October 2014. People with diabetes mellitus were excluded. A total of 321 people were enrolled.

Outcome measures: We divided participants according to tertiles of FI as low, medium, and high levels. Pearson correlation was assessed between insulin level and each of the diagnostic components of metabolic syndrome (MetS-DCs) with adjustment of age. The prevalence of MetS-DCs based on tertiles of FI were studied and analyzed by Cochran-Armitage trend test. The risk for prevalence of MetS in the middle, and high insulin group as compared to the low insulin group were assessed by multivariate logistic regression with adjustments for age, gender, smoking, body mass index (BMI), hypertension, and hyperlipidemia. Youden Index was performed for the optimized cut-off value.

Results: Our results showed positive correlation of FI level with systolic blood pressure, waist circumference, fasting plasma glucose and triglyceride levels, while negative correlation was shown with high-density lipoprotein ($p<0.001$). The prevalence of each MetS-DCs increased as a trend while FI levels increased ($p<0.001$). OR (95% CI) of MetS was 5.04 (2.15-11.81) for high insulin groups compared to the low insulin group after adjusting confounders ($p<0.001$). Area under receiver operating characteristic curve (ROC) curve (AUC) was 0.78, and cut-off value 7.35 $\mu\text{U/mL}$ for FI was obtained (sensitivity: 0.69; specificity: 0.77).

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Conclusions: Middle-aged and elderly non-diabetic people with increased FI are associated with a higher prevalence of MetS. Furthermore, FI is an independent risk factor of MetS.

Keywords: metabolic syndrome, insulin resistance, fasting insulin level

Strengths and limitations of this study:

- This is the first study to explore the relationship between fasting insulin and MetS in the middle-aged and elderly populations of non-diabetic Asian people.
- We offer a biomarker to identify middle-aged and elder non-diabetic Taiwanese with MetS.
- We adjusted many confounding factors to make the results more reliable.
- The recall bias from self-reported lifestyle behaviors is unavoidable.
- The causal relationship between fasting insulin level and MetS cannot be demonstrated in our cross-sectional study.

INTRODUCTION

Metabolic syndrome (MetS) is associated with a cluster of unhealthy metabolic risk factors, including abdominal obesity (excess body fat around the waist), glucose intolerance, pre-morbid hypertension, and dyslipidemia¹⁻³. A number of studies have reported that MetS increases the risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and other non-communicable diseases (NCDs)⁴⁻⁷. The rising prevalence of MetS has created a disturbing challenge to personal health⁸⁻¹².

Insulin resistance has long been associated with MetS¹³⁻¹⁶. Basal insulin represents 45%-50% of daily insulin^{17 18}, and the FI level approximates basal insulin^{18 19}. Studies have shown that FI levels are associated with the prevalence of MetS, which may be due to its representativeness of insulin resistance^{20 21}. A study has even shown that elevated FI levels may predict the future incidence of MetS¹⁹. If insulin resistance is the foundation of MetS^{14 15}, and FI represents insulin resistance with an area under curve (AUC) [95% confidence interval (CI)] of 0.995[0.993-0.996]²⁰, a high FI level may be able to caution the physician for susceptibility to metabolic diseases and hence cardiovascular risks²². We therefore determined the relationships between FI and MetS in middle-aged and elderly populations. In addition, we also obtained a cut-off value for FI as a biomarker for the risk of MetS.

METHODS

Study subjects

This was an observational and cross-sectional study conducted at Linkou Chang Gung Memorial Hospital in Taoyuan County, Taiwan between January and October 2014. The inclusion criteria included residents 50-90 years old and living in Guishan township. 619 residents were eligible for the study. A total of 400 residents agreed to participate in our health exam. Subjects were excluded if they had diabetes. 79 participants with diabetes

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90 mellitus were excluded. Diabetes mellitus was defined as any of the followings: 1. Previous
91 diagnosis of diabetes mellitus; 2. Recent use of oral anti-hyperglycemic drugs or insulin; 3.
92 Participants with fasting glucose \geq 126 mg/dl. A total of 321 participants (111 males and 210
93 females) were ultimately enrolled for analysis. This study was approved by the Institutional
94 Review Board of the hospital and written informed consent was obtained from all of the
95 participants before enrollment.

96 **Data collection**

97 We obtained exercise (exercising \geq 3 times a week or not) and dietary habits (vegetarian
98 or not) from self-administered questionnaires, which also included smoking (current smoker
99 or not) and marital status (currently married or not). Anthropometric data, such as height,
100 weight, waist circumference (WC), and blood pressure were also recorded. The subjects were
101 dressed in light clothing without shoes for weight and height measurements. The BMI was
102 calculated as the weight in kilograms (kg) divided by the height in meters squared (m^2). Waist
103 circumference was measured midway between the inferior margin of the lowest rib and the
104 iliac crest in the horizontal plane while in an upright position. Systolic blood pressure (SBP)
105 and diastolic blood pressure (DBP) were checked at least 2 times after 5 min of rest while
106 seated. FI levels, lipid profile, and fasting glucose were obtained by blood sampling after a
107 10-h overnight fast. Blood samples were analyzed in the central laboratory of Linkou Chang
108 Gung Memorial Hospital for fasting plasma glucose (FPG), serum total cholesterol (TC), low-
109 density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C),
110 serum triglycerides (TG), and FI levels. Serum insulin levels were determined with an
111 ARCHITECT Insulin assay (Abbott Laboratories, IL, USA). Insulin was measured with a
112 chemiluminescent microparticle immunoassay (CMIA). The intra-assay variation and inter-
113 assay variations were less than 2.7%. The ARCHITECT Insulin assay has a sensitivity of
114 $\leq 1.0\mu U/ml$.

Defining MetS

MetS was defined by at least 3 of 5 metabolic syndrome diagnostic components (MetS-DCs), according to The Third Report of the National Cholesterol Education Program Expert Panel (NCEP) on Adult Treatment Panel (ATP III) Asian diagnostic criteria²³. The five MetS-DCs were as follows: 1) SBP \geq 130 mmHg and/or DBP \geq 85 mmHg, or the use of anti-hypertensive drugs; 2) decreased serum HDL-C concentration $<$ 40 mg/dl in men and $<$ 50 mg/dL in women, or treatment for dyslipidemia; 3) TG concentration \geq 150 mg/dl, or on medication for hypertriglyceridemia; 4) hyperglycemia: fasting plasma glucose level \geq 100 mg/dl; and 5) abdominal WC \geq 90 cm in men or \geq 80 cm in women.

Statistical Analysis

Subjects were classified in one of three groups according to serum insulin level tertiles as the low, middle, and high insulin groups. Clinical characteristics were expressed as the mean \pm standard deviation (SD) for continuous variables and number (%) for categorical variables. One-way analysis of variance (ANOVA) or a Chi-square test was used to determine p-values for continuous or categorical variables, respectively. Pearson's correlation was performed for each MetS-DC in relation to FI levels. The Cochran-Armitage trend test was used to evaluate the increasing prevalence of MetS-DCs as a function of insulin level tertile. The low FI group was designated as the reference group to calculate the ORs of the prevalence of MetS in the middle and high FI groups using multivariate logistic regression. Confounded variables present as an obstacle to valid inference in MetS studies. Hypertension and dyslipidemia are both common chronic conditions that affect a large proportion of the general adult population. Previous studies determining the association of FI and MetS also adjusted MetS-DCs¹⁹. Results of the adjusted model provide valid inference among MetS and insulin levels. A ROC curve was created for FI as a biomarker of MetS. The area under the ROC curve (AUC) was analyzed, and the optimized cut-off point for FI, sensitivity, and specificity were acquired

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using the maximal Youden’s Index. We used SPSS (version 23.0 for windows), to perform the statistical analysis. Statistical significance was set at a p-value < 0.05.

RESULTS

A total of 321 individuals, 111 men (34.6%) and 210 (65.4%) women, with a mean age of 63.91±8.32 years, were enrolled in this study. There were 90 study participants (28%) who met the diagnosis of MetS.

Table 1 shows the characteristics of the study population, which was divided based on the FI level in µU/mL. There were no statistically significant differences in age or gender between the low, middle, and high insulin level groups, while differences did exist with respect to WC, SBP, HDL-C, TG, and the proportion with MetS. Table 2 further shows the correlation between the FI level and all MetS-DCs, even after adjusting for age. FI was positively correlated with SBP, WC, FPG, and TG, and negatively correlated with HDL-C, as shown in Table 2. Table 3 shows the prevalence of MetS-DCs (hypertension, hyperglycemia, dyslipidemia, and central obesity) according to the insulin level tertiles. The prevalence of MetS-DCs increased as the FI level increased, as shown by significant p values (Cochran-Armitage trend test). Figure 1 shows that the low insulin level group had a 10% prevalence of MetS, the middle insulin level group had a 21.5% prevalence of MetS, and the high insulin level group had a 53.8% of MetS (p<0.0001 [Cochran-Armitage trend test]), suggesting that the prevalence of MetS increased with an increase in FI levels.

When designating the low insulin level group as the reference, the middle and high insulin level groups had an OR of 2.46 (p=0.02) and 10.50 (p<0.001) for MetS, respectively. After adjusting age, gender, and BMI, the middle and high insulin level groups had an OR of 1.71 (p=0.20) and 5.63 (p<0.001) for MetS, respectively. After adjusting age, gender, BMI, smoking, hypertension and dyslipidemia, the middle and high insulin level groups still had an OR of 1.51 (p=0.35) and 5.04 (p<0.001; Table 4) for MetS, respectively. There was no

significant difference between the middle and low tertile groups, but a significant difference between the high and low tertile groups, even after adjusting for the above risk factors. Based on this data, the high insulin level group had a 5-fold risk for MetS compared to the low insulin level group.

Figure 2 shows the ROC curve of FI as a biomarker for MetS. The AUC was 0.78. The optimized cut-off value for insulin was 7.35 $\mu\text{U/mL}$, with a sensitivity of 0.69 and a specificity of 0.77.

DISCUSSION

In this community-based study, we investigated fasting serum insulin levels in association with the prevalence of MetS in non-diabetic middle-aged and elderly Taiwanese adults. In our study, the prevalence of MetS in the relatively healthy middle age-to-elderly population was 28%, which is similar to the 29.75% findings reported by Li et al.²⁴ Among middle-aged and elderly populations in Taiwan. Looking at the three FI tertiles, there was a rising proportion of MetS as the FI level increased, also shown in previous studies^{20 25 26}. This finding not only applied to MetS, but to MetS-DCs as well. The WC, SBP, TG, and FPG levels were the lowest in the low FI group and highest in the high FI group; the converse applied to HDL and vice versa (Table 1). This finding led us to speculate that an association exists between FI levels and MetS-DCs. We found a statistically significant correlation between FI levels and each MetS-DC, even after adjusting for age (Table 2). A trend existed between the FI level and the prevalence of all five MetS-DCs (Table 3). We thus wanted to know if the trend applied to the prevalence of MetS. Figure 1 shows an apparent increase in the prevalence of MetS as the FI level increased. The trend was confirmed by the Cochran-Armitage trend test ($p < 0.0001$).

After adjusting for gender, age, BMI, smoking status, hypertension, and dyslipidemia, the middle-aged and elderly populations in the high FI group were at significant risk for

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developing MetS (OR = 5.04, 95% CI =2.15-11.81; P<0.01: Table 4). This conclusion is consistent with previous findings^{20 26 27}. Not only is FI an independent risk factor for MetS, but in the cohort study of Sung et al., it was also reported that elevated FI predicted the future incidence of MetS¹⁹. One possible explanation might be the relationship between the FI level and insulin resistance^{20 21}, which has a fundamental role in MetS^{28 29}. Although the mechanism by which FI may represent insulin resistance was not investigated in the present study, a number of studies have shown that FI is a suitable surrogate marker for insulin resistance^{21 30-33}, calculated by the fasting insulin resistance index (FIRI) or homeostasis model assessment of insulin resistance (HOMA-IR). A higher FI level is associated with insulin resistance in patients with impaired fasting glucose, but may be an inappropriate marker in diabetics with poor glycemic control. It has been reported that the FI level is highly associated with MetS²⁰. In our study, the AUC for FI as an indicator for MetS was 0.78, similar to another study's AUC of 0.77²⁰. Based on our search of the literature, there is no widely accepted reference range for FI. A reference range for FI of 1.57–16.32 µU/mL has been proposed in Chinese men, but the reference range varies between different ethnicities and genders³⁴. A FI level above 9µU/mL has been reported to identify 80% of patients with pre-diabetes³⁵. Although we obtained a cut-off value for fasting insulin, but due to large variations in insulin assays, this value of >7.35 should not be generalized to other laboratory sites.

Our findings may have an impact on health screening policies in non-diabetic people older than middle-age. Elevated FI may act as an accompanying marker to enhance the risk of MetS. We do not propose to discard MetS criteria, but suggest that elevated FI may alert physicians on the risk of MetS in clinical settings of non-diabetic individuals. Given the fact that elevated FI is not only associated with a greater risk for developing MetS^{19 36} but is also associated with a greater number of cardiometabolic risk factors²², healthy behavior should

be considered when the FI level is relatively higher in the population. Though we are in need of large trials to determine if subjects with early stages of insulin resistance can benefit from interventions.

Some limitations in our study merit consideration. First, the principal limitation relevant to the interpretation of our results was the use of a cross-sectional design, thus a causal relationship between the FI level and MetS cannot be inferred. Second, the sample size in our study was relatively small ($n=321$ [the power was not calculated]) and the participants were recruited from a regional community. The participants could therefore only be distributed into three groups and the results cannot be generalized to other ethnicities. Third, though males tend to have a lower participation rate in studies³⁷, there may still have been a selection bias due to the higher participation of women than men in our study. Fourth, the FI cutoff value varies between different ethnic groups and insulin assays, so physicians should be aware of this variation in clinical settings. Besides, the false negative rate (31%) should be taken into consideration when applying this data. Furthermore, even though we used a standardized questionnaire, recall and reporting bias are unavoidable for self-reported data. Finally, we did not ask participants to sleep adequately or to avoid vigorous exercise the day before blood testing, which could affect the accuracy of the fasting serum insulin level.

Our study also has strengths. First, our participants were recruited during a community health examination and represent a relatively healthy population. The effects of important confounders, including ethnicity, residential area, and environmental factors, were minimized. Second, we used standardized laboratory examination protocols and anthropometric measurements. Third, while evaluating the association between MetS and the FI level, we excluded diabetic patients to avoid the effect of anti-diabetic medications on the FI level. Lastly, due to the trend of world aging, our study aimed for middle-aged and elderly populations. Studies from all around the world indicate the relationship of FI and MetS (table

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240 5), our study contributes to the Taiwanese population.

241 In the future, we will continue to follow this community and record the development of
242 newly diagnosed MetS. Counseling of healthy behaviors for residents with elevated FI will
243 also be our topic of interest hereon. Whether lifestyle modification could retard the
244 development of MetS in high FI individuals requires further studies to elaborate.

245 **CONCLUSIONS**

246 Our study provides a method to identify the risk of MetS by testing FI levels in the
247 middle aged and elderly non-diabetic populations. When a non-diabetic individual is
248 presented with a high FI level, physicians may be alerted of the risk of MetS. Our study
249 confirms the association between FI and MetS. Further prospective research is needed to
250 clarify the link between FI and MetS.

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253 **Contributors** YHC and YCL were involved in writing of the manuscript. YCT, MCL, HHC
254 and WCY conceived and supervised the study. IST provided statistical advice. JYC
255 contributed conceived, designed and performed the experiments, collected and analyzed the
256 data, revising it critically for important intellectual content and final approval of the version to
257 be submitted.

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260 **Competing interests** None declared.

261 **Ethics approval** The study was approved by Chang-Gung Medical Foundation Institutional
262 Review Board (102-2304B), and written informed consent was given by all the participants
263 before enrollment.

264 **Data sharing statement** No additional data are available.

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Figure Legends

Figure 1. Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

Figure 2. ROC curve for insulin as a biomarker of metabolic syndrome. AUC 0.78. FI 7.35µU/mL (sensitivity: 0.69; specificity: 0.77).

Table 1 General characteristics of the study population based on insulin levels.

Variables	Insulin levels				p value
	Total n=321	Low n=110 (≤4.8)	Middle n=107 (4.9-7.8)	High n=104 (≥7.9)	
Age (year)	63.91 ±8.32	64.23 ±8.32	64.47 ±8.67	63.01 ±7.93	0.40
BMI (kg/m ²)	24.36 ±3.53	22.41 ±3.14	24.41 ±2.73	26.37 ±3.54	<0.001
Waist circumference (cm)	84.23 ±9.51	79.69 ±7.57	83.78 ±8.63	89.51 ±9.65	<0.001
SBP (mmHg)	129.02 ±16.59	123.69 ±17.36	129.52 ±14.51	134.13 ±16.20	<0.001
DBP (mmHg)	77.01 ±10.90	75.43 ±11.80	76.92 ±10.03	78.79 ±10.60	0.08
ALT (U/L)	21.74 ±11.06	18.94 ±7.81	20.33 ±9.25	26.15 ±14.05	<0.001
Creatinine (mg/dL)	0.76 ±0.44	0.69 ±0.17	0.85 ±0.66	0.75 ±0.34	0.03
FPG (mg/dL)	89.10 ±9.93	85.29 ±9.11	89.18 ±8.52	93.05 ±10.60	<0.001
HDL-C (mg/dL)	55.70 ±14.05	60.93 ±14.85	55.59 ±13.17	50.28 ±11.94	<0.001
Insulin (μU/mL)	7.10 ±4.14	3.60 ±0.94	6.21 ±0.86	11.72 ±4.02	<0.001
LDL-C (mg/dL)	121.48 ±32.05	118.90 ±34.55	126.03 ±31.01	119.53 ±30.10	0.20
T-cholesterol (mg/dL)	200.61 ±35.20	198.85 ±36.98	203.81 ±35.12	119.18 ±33.43	0.52
TG (mg/dL)	117.34 ±60.61	95.39 ±45.13	111.04 ±49.83	147.05 ±72.48	<0.001
Current smoking, n(%)	34 (10.6)	14 (12.7)	11 (10.3)	9 (8.7)	0.62
Marital status (single), n(%)	54 (16.8)	22 (20.0)	14 (13.1)	18 (17.3)	0.39
Men, n(%)	111 (34.6)	41 (37.3)	39 (36.4)	31 (29.8)	0.46
Regular exercise, n(%)	264 (82.2)	92 (83.6)	96 (89.7)	76 (73.1)	0.01
Vegetarian, n(%)	20 (6.2)	7 (6.4)	7 (6.5)	6 (5.8)	0.97
HTN, n(%)	150 (46.7)	43 (39.1)	47 (43.9)	60 (57.7)	0.02
Hyperlipidemia, n(%)	204 (63.6)	58 (52.7)	69 (64.5)	77 (74.0)	0.005
Metabolic syndrome, n(%)	90 (28.0)	11 (10.0)	23 (21.5)	56 (53.8)	<0.001

Notes: Ranges of FI levels of different tertile groups are shown in brackets at the top of the table, units in μU/mL.

Clinical characteristics are expressed as the mean±SD for continuous variables and n(%) for categorical variables. P-values were derived from one-way analysis of variance (ANOVA) for

continuous variables and chi-square test for categorical variables.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HTN, hypertension.

Table 2 Pearson's correlation coefficients for each component of metabolic syndrome and age in relation to insulin levels.

Variables	Insulin(n=321)			
	Unadjusted		Adjusted for age	
	Pearson's coefficient	p value	Pearson's coefficient	p value
Age (year)	-0.04	0.50	NA	NA
SBP (mmHg)	0.21	<0.001	0.22	<0.001
DBP (mmHg)	0.11	0.05	0.10	0.07
Waist circumference (cm)	0.43	<0.001	0.44	<0.001
FPG (mg/dL)	0.38	<0.001	0.39	<0.001
HDL-C (mg/dL)	-0.37	<0.001	-0.37	<0.001
TG (mg/dL)	0.37	<0.001	0.37	<0.001

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

Table 3 Prevalence of components of metabolic syndrome based on insulin levels.

Components	Low (n=110) N(%)	Middle (n=107) N(%)	High (n=104) N(%)	p value for Cochran- Armitage trend test
High blood pressure ^a	56(50.9)	63(58.9)	78(75)	0.0003
High blood glucose ^b	8(7.3)	10(9.3)	25(24.0)	0.0004
Low HDL-C ^c	15(13.6)	19(17.8)	43(41.3)	<0.0001
High TG ^d	17(15.5)	27(25.2)	42(40.4)	<0.0001
Central obesity ^e	34(30.9)	57(53.3)	82(78.8)	<0.0001

Note:

^a SBP \geq 130 mmHg or DBP \geq 85 mmHg, or self-reported hypertension

^b Fasting blood glucose \geq 100 mg/dL or self-reported diabetes mellitus

^c HDL-C<40 mg/dL in men or <50 mg/dL in women

^d TG \geq 150 mg/dL

^e Waist circumference \geq 90 cm in men or \geq 80 cm in women

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 4 Association between insulin levels and metabolic syndrome.

Variables	Model 1			Model 2			Model 3		
	OR	(95%CI)	p value	OR	(95%CI)	p value	OR	(95%CI)	p value
Low	1.00	—	—	1.00	—	—	1.00	—	—
Middle	2.46	(1.14-5.35)	0.02	1.71	(0.76-3.85)	0.20	1.51	(0.64-3.57)	0.35
High	10.50	(5.05-21.84)	<0.001	5.63	(2.53-12.53)	<0.001	5.04	(2.15-11.81)	<0.001
p value for trend			<0.001			<0.001			<0.001

Model 1: unadjusted

Model 2: adjusted for gender, age, and BMI

Model 3: adjusted for factors in model 2 plus smoking, HTN, and hyperlipidemia

Abbreviations: BMI, body mass index; HTN, hypertension; OR, odds ratio; CI, confidence interval.

427 **Table 5** Studies of Association Between Fasting Insulin and Metabolic Syndrome.

Authors	Study Year	Study Population	% MetS	Fasting insulin	Risk of MetS	Main Finding	Refer ence
Saravia G et al 2015	2009-2010 Cross sectional	3200 Non- diabetic males in Spain	23%	Highest tertile (≥6.13) vs lowest (≤3.80)μU/mL	OR (95%CI) 11.36 (8.65- 15.13) for MetS	Per each 10 pmol/L (1.4 uU/mL) increase in insulin, the odds for metabolic syndrome increased by 1.43 (95%CI: 1.38, 1.49)	[20]
Rutter MK et al 2014	(1991-1995) to (1998-2001) 7 year Prospective	2616 non- diabetic adults in Europe	-	1-quintile change in fasting insulin (pmol/L)	mean (95% CI) 0.12 (0.10–0.15) [MetS trait score 7 year change]	Change in metabolic trait clustering was significantly associated with baseline levels and changes in fasting insulin.	[38]
Sung KC et al 2011	2003-2008 5 year cohort	2350 non- MetS in Korea	8.5% (incid ence)	Highest quartile (≥8.98) vs lowest (≤6.01) IU/ml	OR (95% CI) of developing MS 5.1 (3.1-8.2)	The highest quartile of the insulin levels had more than a 5 times greater risk of developing MS compared to the subjects in the lowest quartile.	[19]
Kanda H et al 2011	2000,2001 Cross sectional	456 in Mongolia	6.4%	Highest tertile (≥10.33) vs lowest (≤6.72) mmol/L	Percentage of MetS 17.1% vs 4.6%	Fasting plasma insulin is associated with MetS in farmers, but not nomads among the Mongolian population in China.	[27]
STOPP- T2D PSG* 2008	2003 Cross sectional	1453 8 th grade adolescents in the U.S.	9.5%	Highest quintile (≥39.1) vs lowest (≤17.0) μU/mL	OR (95%CI) 199.64(31.29- 1273.7) for MetS	The highest insulin quintile were almost 200 times more likely to be classified with the metabolic syndrome than participants in the lowest quintile.	[28]
Adam FM et al 2006	2005 Cross sectional	128 overweight/ obese in Indonesia	68.8%	Mean fasting insulin levels15.68±7.85 vs 3.16±2.53 (uU/ml) with 5 components vs 1 component of MetS.		There is a strong linear increase in fasting insulin levels with an increase of the number of metabolic syndrome.	[29]

428 *STOPP-T2D PSG: Studies to Treat or Prevent Pediatric Type 2 Diabetes Prevention Study Group

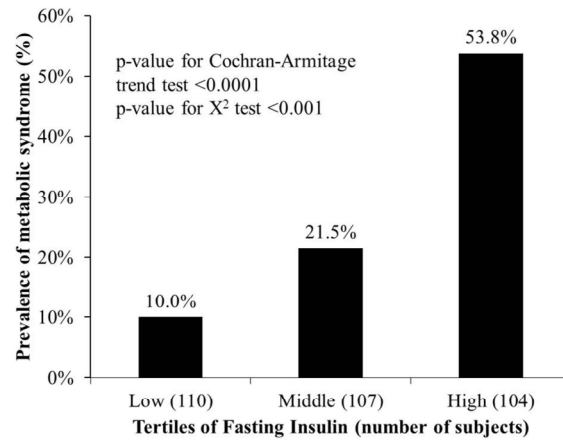


Figure 1 Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

Figure 1. Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

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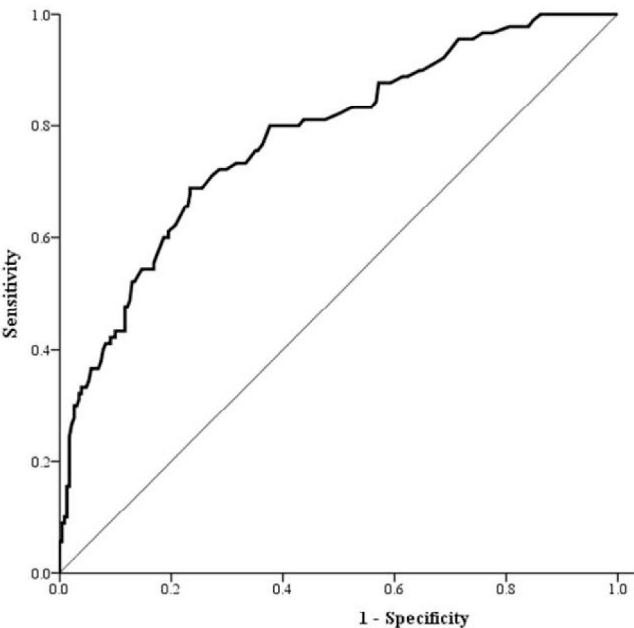


Figure 2. ROC curve for insulin as a biomarker of metabolic syndrome. AUC 0.78. FI 7.35 μ U/mL (sensitivity: 0.69; specificity: 0.77).

Figure 2. ROC curve for insulin as a biomarker of metabolic syndrome. AUC 0.78. FI 7.35 μ U/mL (sensitivity: 0.69; specificity: 0.77).

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	P2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	P2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any prespecified hypotheses	P2, P4
Methods			
Study design	4	Present key elements of study design early in the paper	P4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P4, 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	P4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	Nil
Study size	10	Explain how the study size was arrived at	Nil
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	P6
		(b) Describe any methods used to examine subgroups and interactions	P6
		(c) Explain how missing data were addressed	Nil
		(d) If applicable, describe analytical methods taking account of sampling strategy	Nil
		(e) Describe any sensitivity analyses	Nil
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Nil
		(b) Give reasons for non-participation at each stage	Nil
		(c) Consider use of a flow diagram	Nil
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P8
		(b) Indicate number of participants with missing data for each variable of interest	Nil
Outcome data	15*	Report numbers of outcome events or summary measures	P6-9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P7
		(b) Report category boundaries when continuous variables were categorized	P8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Nil
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Nil
Discussion			
Key results	18	Summarise key results with reference to study objectives	P7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P17
Generalisability	21	Discuss the generalisability (external validity) of the study results	P18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between high fasting insulin levels and metabolic syndrome in non-diabetic middle-aged and elderly populations: A community-based study in Taiwan

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Secondary Subject Heading:	General practice / Family practice, Geriatric medicine, Health policy
Keywords:	metabolic syndrome, insulin resistance, fasting insulin level

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1 ● **Title**

2 Association between high fasting insulin levels and metabolic syndrome in non-diabetic
3 middle-aged and elderly populations: A community-based study in Taiwan

4 ● **Short title**

5 Increased fasting insulin levels in association with metabolic syndrome

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21 ● **Format**

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26

ABSTRACT

Objectives: We aimed to determine the association between fasting insulin (FI) levels and metabolic syndrome (MetS) in non-diabetic middle-aged and elderly adults in a community in Taiwan.

Design: Cross-sectional observational study.

Setting: Community-based investigation in Guishan township of northern Taiwan.

Participants: Our study included adults aged 50 years and above during community health exams between January and October 2014. People with diabetes mellitus were excluded. A total of 321 people were enrolled.

Outcome measures: We divided participants according to tertiles of FI as low, medium, and high levels. Pearson correlation was assessed between insulin level and each of the diagnostic components of metabolic syndrome (MetS-DCs) with adjustment of age. The prevalence of MetS-DCs based on tertiles of FI were studied and analyzed by Cochran-Armitage trend test. The risk for prevalence of MetS in the middle, and high insulin group as compared to the low insulin group were assessed by multivariate logistic regression with adjustments for age, gender, smoking, body mass index (BMI), hypertension, and hyperlipidemia. Youden Index was performed for the optimized cut-off value.

Results: Our results showed positive correlation of FI level with systolic blood pressure, waist circumference, fasting plasma glucose and triglyceride levels, while negative correlation was shown with high-density lipoprotein ($p < 0.001$). The prevalence of each MetS-DCs increased as a trend while FI levels increased ($p < 0.001$). OR (95% CI) of MetS was 5.04 (2.15-11.81) for high insulin groups compared to the low insulin group after adjusting confounders ($p < 0.001$). Area under receiver operating characteristic curve (ROC) curve (AUC) was 0.78, and cut-off value 7.35 $\mu\text{U/mL}$ for FI was obtained (sensitivity: 0.69; specificity: 0.77).

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Conclusions: Middle-aged and elderly non-diabetic people with increased FI are associated with a higher prevalence of MetS in the community in Taiwan. Furthermore, FI is an independent risk factor of MetS in this study population.

Keywords: metabolic syndrome, insulin resistance, fasting insulin level

Strengths and limitations of this study:

- This is the first study to explore the relationship between fasting insulin and MetS in the middle-aged and elderly populations of non-diabetic Asian people.
- We offer a biomarker to identify middle-aged and elder non-diabetic Taiwanese with MetS.
- We adjusted many confounding factors to make the results more reliable.
- The recall bias from self-reported lifestyle behaviors is unavoidable.
- The causal relationship between fasting insulin level and MetS cannot be demonstrated in our cross-sectional study.

INTRODUCTION

Metabolic syndrome (MetS) is associated with a cluster of unhealthy metabolic risk factors, including abdominal obesity (excess body fat around the waist), glucose intolerance, pre-morbid hypertension, and dyslipidemia¹⁻³. A number of studies have reported that MetS increases the risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and other non-communicable diseases (NCDs)⁴⁻⁷. The rising prevalence of MetS has created a disturbing challenge to personal health⁸⁻¹².

Insulin resistance has long been associated with MetS¹³⁻¹⁶. Basal insulin represents 45%-50% of daily insulin^{17 18}, and the FI level approximates basal insulin^{18 19}. Studies have shown that FI levels are associated with the prevalence of MetS, which may be due to its representativeness of insulin resistance^{20 21}. A study has even shown that elevated FI levels may predict the future incidence of MetS¹⁹. If insulin resistance is the foundation of MetS^{14 15}, and FI represents insulin resistance with an area under curve (AUC) [95% confidence interval (CI)] of 0.995[0.993-0.996]²⁰, a high FI level may be able to caution the physician for susceptibility to metabolic diseases and hence cardiovascular risks²². We therefore aimed to determine the association between FI levels and MetS in non-diabetic middle-aged and elderly adults in a community in Taiwan.

METHODS

Study subjects

This was an observational and cross-sectional study conducted at Linkou Chang Gung Memorial Hospital in Taoyuan County, Taiwan between January and October 2014. The inclusion criteria included residents 50-90 years old and living in Guishan township. 619 residents were eligible for the study. A total of 400 residents agreed to participate in our health exam. Subjects were excluded if they had diabetes. 79 participants with diabetes

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90 mellitus were excluded. Diabetes mellitus was defined as any of the followings: 1. Previous
91 diagnosis of diabetes mellitus; 2. Recent use of oral anti-hyperglycemic drugs or insulin; 3.
92 Participants with fasting glucose \geq 126 mg/dl. A total of 321 participants (111 males and 210
93 females) were ultimately enrolled for analysis. This study was approved by the Institutional
94 Review Board of the hospital and written informed consent was obtained from all of the
95 participants before enrollment.

96 **Data collection**

97 We obtained exercise (exercising \geq 3 times a week or not) and dietary habits (vegetarian
98 or not) from self-administered questionnaires, which also included smoking (current smoker
99 or not) and marital status (currently married or not). Anthropometric data, such as height,
100 weight, waist circumference (WC), and blood pressure were also recorded. The subjects were
101 dressed in light clothing without shoes for weight and height measurements. The BMI was
102 calculated as the weight in kilograms (kg) divided by the height in meters squared (m^2). Waist
103 circumference was measured midway between the inferior margin of the lowest rib and the
104 iliac crest in the horizontal plane while in an upright position. Systolic blood pressure (SBP)
105 and diastolic blood pressure (DBP) were checked at least 2 times after 5 min of rest while
106 seated. FI levels, lipid profile, and fasting glucose were obtained by blood sampling after a
107 10-h overnight fast. Blood samples were analyzed in the central laboratory of Linkou Chang
108 Gung Memorial Hospital for fasting plasma glucose (FPG), serum total cholesterol (TC), low-
109 density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C),
110 serum triglycerides (TG), and FI levels. Serum insulin levels were determined with an
111 ARCHITECT Insulin assay (Abbott Laboratories, IL, USA). Insulin was measured with a
112 chemiluminescent microparticle immunoassay (CMIA). The intra-assay variation and inter-
113 assay variations were less than 2.7%. The ARCHITECT Insulin assay has a sensitivity of
114 $\leq 1.0\mu U/ml$.

Defining MetS

MetS was defined by at least 3 of 5 metabolic syndrome diagnostic components (MetS-DCs), according to The Third Report of the National Cholesterol Education Program Expert Panel (NCEP) on Adult Treatment Panel (ATP III) Asian diagnostic criteria²³. The five MetS-DCs were as follows: 1) SBP \geq 130 mmHg and/or DBP \geq 85 mmHg, or the use of anti-hypertensive drugs; 2) decreased serum HDL-C concentration $<$ 40 mg/dl in men and $<$ 50 mg/dL in women, or treatment for dyslipidemia; 3) TG concentration \geq 150 mg/dl, or on medication for hypertriglyceridemia; 4) hyperglycemia: fasting plasma glucose level \geq 100 mg/dl; and 5) abdominal WC \geq 90 cm in men or \geq 80 cm in women.

Statistical Analysis

Subjects were classified in one of three groups according to serum insulin level tertiles as the low, middle, and high insulin groups. Clinical characteristics were expressed as the mean \pm standard deviation (SD) for continuous variables and number (%) for categorical variables. One-way analysis of variance (ANOVA) or a Chi-square test was used to determine p-values for continuous or categorical variables, respectively. Pearson's correlation was performed for each MetS-DC in relation to FI levels. The Cochran-Armitage trend test was used to evaluate the increasing prevalence of MetS-DCs as a function of insulin level tertile. The low FI group was designated as the reference group to calculate the ORs of the prevalence of MetS in the middle and high FI groups using multivariate logistic regression. Confounded variables present as an obstacle to valid inference in MetS studies. Hypertension and dyslipidemia are both common chronic conditions that affect a large proportion of the general adult population. Previous studies determining the association of FI and MetS also adjusted MetS-DCs¹⁹. Results of the adjusted model provide valid inference among MetS and insulin levels. A ROC curve was created for FI as a biomarker of MetS. The area under the ROC curve (AUC) was analyzed, and the optimized cut-off point for FI, sensitivity, and specificity were acquired

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using the maximal Youden’s Index. We used SPSS (version 23.0 for windows), to perform the statistical analysis. Statistical significance was set at a p-value < 0.05.

RESULTS

A total of 321 individuals, 111 men (34.6%) and 210 (65.4%) women, with a mean age of 63.91±8.32 years, were enrolled in this study. There were 90 study participants (28%) who met the diagnosis of MetS.

Table 1 shows the characteristics of the study population, which was divided based on the FI level in µU/mL. There were no statistically significant differences in age or gender between the low, middle, and high insulin level groups, while differences did exist with respect to WC, SBP, HDL-C, TG, and the proportion with MetS. Table 2 further shows the correlation between the FI level and all MetS-DCs, even after adjusting for age. FI was positively correlated with SBP, WC, FPG, and TG, and negatively correlated with HDL-C, as shown in Table 2. Table 3 shows the prevalence of MetS-DCs (hypertension, hyperglycemia, dyslipidemia, and central obesity) according to the insulin level tertiles. The prevalence of MetS-DCs increased as the FI level increased, as shown by significant p values (Cochran-Armitage trend test). Figure 1 shows that the low insulin level group had a 10% prevalence of MetS, the middle insulin level group had a 21.5% prevalence of MetS, and the high insulin level group had a 53.8% of MetS (p<0.0001 [Cochran-Armitage trend test]), suggesting that the prevalence of MetS increased with an increase in FI levels.

When designating the low insulin level group as the reference, the middle and high insulin level groups had an OR of 2.46 (p=0.02) and 10.50 (p<0.001) for MetS, respectively. After adjusting age, gender, and BMI, the middle and high insulin level groups had an OR of 1.71 (p=0.20) and 5.63 (p<0.001) for MetS, respectively. After adjusting age, gender, BMI, smoking, hypertension and dyslipidemia, the middle and high insulin level groups still had an OR of 1.51 (p=0.35) and 5.04 (p<0.001; Table 4) for MetS, respectively. There was no

significant difference between the middle and low tertile groups, but a significant difference between the high and low tertile groups, even after adjusting for the above risk factors. Based on this data, the high insulin level group had a 5-fold risk for MetS compared to the low insulin level group.

Figure 2 shows the ROC curve of FI as a biomarker for MetS. The AUC was 0.78. The optimized cut-off value for insulin was 7.35 $\mu\text{U/mL}$, with a sensitivity of 0.69 and a specificity of 0.77.

DISCUSSION

In this community-based study, we investigated fasting serum insulin levels in association with the prevalence of MetS in non-diabetic middle-aged and elderly Taiwanese adults. In our study, the prevalence of MetS in the relatively healthy middle age-to-elderly population was 28%, which is similar to the 29.75% findings reported by Li et al.²⁴ Among middle-aged and elderly populations in Taiwan. Looking at the three FI tertiles, there was a rising proportion of MetS as the FI level increased, also shown in previous studies^{20 25 26}. This finding not only applied to MetS, but to MetS-DCs as well. The WC, SBP, TG, and FPG levels were the lowest in the low FI group and highest in the high FI group; the converse applied to HDL and vice versa (Table 1). This finding led us to speculate that an association exists between FI levels and MetS-DCs. We found a statistically significant correlation between FI levels and each MetS-DC, even after adjusting for age (Table 2). A trend existed between the FI level and the prevalence of all five MetS-DCs (Table 3). We thus wanted to know if the trend applied to the prevalence of MetS. Figure 1 shows an apparent increase in the prevalence of MetS as the FI level increased. The trend was confirmed by the Cochran-Armitage trend test ($p < 0.0001$).

After adjusting for gender, age, BMI, smoking status, hypertension, and dyslipidemia, the middle-aged and elderly populations in the high FI group were at significant risk for

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developing MetS (OR = 5.04, 95% CI =2.15-11.81; P<0.01: Table 4). This conclusion is consistent with previous findings^{20 26 27}. Not only is FI an independent risk factor for MetS, but in the cohort study of Sung et al., it was also reported that elevated FI predicted the future incidence of MetS¹⁹. One possible explanation might be the relationship between the FI level and insulin resistance^{20 21}, which has a fundamental role in MetS^{28 29}. Although the mechanism by which FI may represent insulin resistance was not investigated in the present study, a number of studies have shown that FI is a suitable surrogate marker for insulin resistance^{21 30-33}, calculated by the fasting insulin resistance index (FIRI) or homeostasis model assessment of insulin resistance (HOMA-IR). A higher FI level is associated with insulin resistance in patients with impaired fasting glucose, but may be an inappropriate marker in diabetics with poor glycemic control. It has been reported that the FI level is highly associated with MetS²⁰. In our study, the AUC for FI as an indicator for MetS was 0.78, similar to another study's AUC of 0.77²⁰. Based on our search of the literature, there is no widely accepted reference range for FI. A reference range for FI of 1.57–16.32 µU/mL has been proposed in Chinese men, but the reference range varies between different ethnicities and genders³⁴. A FI level above 9µU/mL has been reported to identify 80% of patients with pre-diabetes³⁵. Although we obtained a cut-off value for fasting insulin, but due to large variations in insulin assays, this value of >7.35 should not be generalized to other laboratory sites.

Our findings may have an impact on health screening policies in non-diabetic people older than middle-age. Elevated FI may act as an accompanying marker to enhance the risk of MetS. We do not propose to discard MetS criteria, but suggest that elevated FI may alert physicians on the risk of MetS in clinical settings of non-diabetic individuals. Given the fact that elevated FI is not only associated with a greater risk for developing MetS^{19 36} but is also associated with a greater number of cardiometabolic risk factors²², healthy behavior should

be considered when the FI level is relatively higher in the population. Though we are in need of large trials to determine if subjects with early stages of insulin resistance can benefit from interventions.

Some limitations in our study merit consideration. First, the principal limitation relevant to the interpretation of our results was the use of a cross-sectional design, thus a causal relationship between the FI level and MetS cannot be inferred. Second, the sample size in our study was relatively small ($n=321$ [the power was not calculated]) and the participants were recruited from a regional community. The participants could therefore only be distributed into three groups and the results cannot be generalized to other ethnicities. Third, though males tend to have a lower participation rate in studies³⁷, there may still have been a selection bias due to the higher participation of women than men in our study. Fourth, the FI cutoff value varies between different ethnic groups and insulin assays, so physicians should be aware of this variation in clinical settings. Besides, the false negative rate (31%) should be taken into consideration when applying this data. Furthermore, even though we used a standardized questionnaire, recall and reporting bias are unavoidable for self-reported data. Finally, we did not ask participants to sleep adequately or to avoid vigorous exercise the day before blood testing, which could affect the accuracy of the fasting serum insulin level.

Our study also has strengths. First, our participants were recruited during a community health examination and represent a relatively healthy population. The effects of important confounders, including ethnicity, residential area, and environmental factors, were minimized. Second, we used standardized laboratory examination protocols and anthropometric measurements. Third, while evaluating the association between MetS and the FI level, we excluded diabetic patients to avoid the effect of anti-diabetic medications on the FI level. Lastly, due to the trend of world aging, our study aimed for middle-aged and elderly populations. Studies from all around the world indicate the relationship of FI and MetS (table

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240 5), our study contributes to the Taiwanese population.

241 In the future, we will continue to follow this community and record the development of
242 newly diagnosed MetS. Counseling of healthy behaviors for residents with elevated FI will
243 also be our topic of interest hereon. Whether lifestyle modification could retard the
244 development of MetS in high FI individuals requires further studies to elaborate.

245 **CONCLUSIONS**

246 Our study provides a method to identify the risk of MetS by testing FI levels in the
247 middle aged and elderly non-diabetic populations. When a non-diabetic individual is
248 presented with a high FI level, physicians may be alerted of the risk of MetS. Our study
249 confirms the association between FI and MetS. Further prospective research is needed to
250 clarify the link between FI and MetS.

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252 **Contributors** YHC and YCL were involved in writing of the manuscript. YCT, MCL, HHC
253 and WCY conceived and supervised the study. IST provided statistical advice. JYC
254 contributed conceived, designed and performed the experiments, collected and analyzed the
255 data, revising it critically for important intellectual content and final approval of the version to
256 be submitted.

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259 **Competing interests** None declared.

260 **Ethics approval** The study was approved by Chang-Gung Medical Foundation Institutional
261 Review Board (102-2304B), and written informed consent was given by all the participants
262 before enrollment.

263 **Data sharing statement** No additional data are available.

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Figure Legends

Figure 1. Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend
across insulin tertiles.

Figure 2. ROC curve for insulin as a biomarker of metabolic syndrome. AUC 0.78. FI
7.35µU/mL (sensitivity: 0.69; specificity: 0.77).

Table 1 General characteristics of the study population based on insulin levels.

Variables	Insulin levels				p value
	Total n=321	Low n=110 (≤4.8)	Middle n=107 (4.9-7.8)	High n=104 (≥7.9)	
Age (year)	63.91 ±8.32	64.23 ±8.32	64.47 ±8.67	63.01 ±7.93	0.40
BMI (kg/m ²)	24.36 ±3.53	22.41 ±3.14	24.41 ±2.73	26.37 ±3.54	<0.001
Waist circumference (cm)	84.23 ±9.51	79.69 ±7.57	83.78 ±8.63	89.51 ±9.65	<0.001
SBP (mmHg)	129.02 ±16.59	123.69 ±17.36	129.52 ±14.51	134.13 ±16.20	<0.001
DBP (mmHg)	77.01 ±10.90	75.43 ±11.80	76.92 ±10.03	78.79 ±10.60	0.08
ALT (U/L)	21.74 ±11.06	18.94 ±7.81	20.33 ±9.25	26.15 ±14.05	<0.001
Creatinine (mg/dL)	0.76 ±0.44	0.69 ±0.17	0.85 ±0.66	0.75 ±0.34	0.03
FPG (mg/dL)	89.10 ±9.93	85.29 ±9.11	89.18 ±8.52	93.05 ±10.60	<0.001
HDL-C (mg/dL)	55.70 ±14.05	60.93 ±14.85	55.59 ±13.17	50.28 ±11.94	<0.001
Insulin (μU/mL)	7.10 ±4.14	3.60 ±0.94	6.21 ±0.86	11.72 ±4.02	<0.001
LDL-C (mg/dL)	121.48 ±32.05	118.90 ±34.55	126.03 ±31.01	119.53 ±30.10	0.20
T-cholesterol (mg/dL)	200.61 ±35.20	198.85 ±36.98	203.81 ±35.12	119.18 ±33.43	0.52
TG (mg/dL)	117.34 ±60.61	95.39 ±45.13	111.04 ±49.83	147.05 ±72.48	<0.001
Current smoking, n(%)	34 (10.6)	14 (12.7)	11 (10.3)	9 (8.7)	0.62
Marital status (single), n(%)	54 (16.8)	22 (20.0)	14 (13.1)	18 (17.3)	0.39
Men, n(%)	111 (34.6)	41 (37.3)	39 (36.4)	31 (29.8)	0.46
Regular exercise, n(%)	264 (82.2)	92 (83.6)	96 (89.7)	76 (73.1)	0.01
Vegetarian, n(%)	20 (6.2)	7 (6.4)	7 (6.5)	6 (5.8)	0.97
HTN, n(%)	150 (46.7)	43 (39.1)	47 (43.9)	60 (57.7)	0.02
Hyperlipidemia, n(%)	204 (63.6)	58 (52.7)	69 (64.5)	77 (74.0)	0.005
Metabolic syndrome, n(%)	90 (28.0)	11 (10.0)	23 (21.5)	56 (53.8)	<0.001

Notes: Ranges of FI levels of different tertile groups are shown in brackets at the top of the table, units in μU/mL.

Clinical characteristics are expressed as the mean±SD for continuous variables and n(%) for categorical variables. P-values were derived from one-way analysis of variance (ANOVA) for

continuous variables and chi-square test for categorical variables.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HTN, hypertension.

Table 2 Pearson's correlation coefficients for each component of metabolic syndrome and age in relation to insulin levels.

Variables	Insulin(n=321)			
	Unadjusted		Adjusted for age	
	Pearson's coefficient	p value	Pearson's coefficient	p value
Age (year)	-0.04	0.50	NA	NA
SBP (mmHg)	0.21	<0.001	0.22	<0.001
DBP (mmHg)	0.11	0.05	0.10	0.07
Waist circumference (cm)	0.43	<0.001	0.44	<0.001
FPG (mg/dL)	0.38	<0.001	0.39	<0.001
HDL-C (mg/dL)	-0.37	<0.001	-0.37	<0.001
TG (mg/dL)	0.37	<0.001	0.37	<0.001

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

Table 3 Prevalence of components of metabolic syndrome based on insulin levels.

Components	Low (n=110) N(%)	Middle (n=107) N(%)	High (n=104) N(%)	p value for Cochran- Armitage trend test
High blood pressure ^a	56(50.9)	63(58.9)	78(75)	0.0003
High blood glucose ^b	8(7.3)	10(9.3)	25(24.0)	0.0004
Low HDL-C ^c	15(13.6)	19(17.8)	43(41.3)	<0.0001
High TG ^d	17(15.5)	27(25.2)	42(40.4)	<0.0001
Central obesity ^e	34(30.9)	57(53.3)	82(78.8)	<0.0001

Note:

^a SBP \geq 130 mmHg or DBP \geq 85 mmHg, or self-reported hypertension

^b Fasting blood glucose \geq 100 mg/dL or self-reported diabetes mellitus

^c HDL-C<40 mg/dL in men or <50 mg/dL in women

^d TG \geq 150 mg/dL

^e Waist circumference \geq 90 cm in men or \geq 80 cm in women

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 4 Association between insulin levels and metabolic syndrome.

Variables	Model 1			Model 2			Model 3		
	OR	(95%CI)	p value	OR	(95%CI)	p value	OR	(95%CI)	p value
Low	1.00	—	—	1.00	—	—	1.00	—	—
Middle	2.46	(1.14-5.35)	0.02	1.71	(0.76-3.85)	0.20	1.51	(0.64-3.57)	0.35
High	10.50	(5.05-21.84)	<0.001	5.63	(2.53-12.53)	<0.001	5.04	(2.15-11.81)	<0.001
p value for trend			<0.001			<0.001			<0.001

Model 1: unadjusted

Model 2: adjusted for gender, age, and BMI

Model 3: adjusted for factors in model 2 plus smoking, HTN, and hyperlipidemia

Abbreviations: BMI, body mass index; HTN, hypertension; OR, odds ratio; CI, confidence interval.

427 **Table 5** Studies of Association Between Fasting Insulin and Metabolic Syndrome.

Authors	Study Year	Study Population	% MetS	Fasting insulin	Risk of MetS	Main Finding	Refer ence
Saravia G et al 2015	2009-2010 Cross sectional	3200 Non- diabetic males in Spain	23%	Highest tertile (≥6.13) vs lowest (≤3.80)μU/mL	OR (95%CI) 11.36 (8.65- 15.13) for MetS	Per each 10 pmol/L (1.4 uU/mL) increase in insulin, the odds for metabolic syndrome increased by 1.43 (95%CI: 1.38, 1.49)	[20]
Rutter MK et al 2014	(1991-1995) to (1998-2001) 7 year Prospective	2616 non- diabetic adults in Europe	-	1-quintile change in fasting insulin (pmol/L)	mean (95% CI) 0.12 (0.10–0.15) [MetS trait score 7 year change]	Change in metabolic trait clustering was significantly associated with baseline levels and changes in fasting insulin.	[38]
Sung KC et al 2011	2003-2008 5 year cohort	2350 non- MetS in Korea	8.5% (incid ence)	Highest quartile (≥8.98) vs lowest (≤6.01) IU/ml	OR (95% CI) of developing MS 5.1 (3.1-8.2)	The highest quartile of the insulin levels had more than a 5 times greater risk of developing MS compared to the subjects in the lowest quartile.	[19]
Kanda H et al 2011	2000,2001 Cross sectional	456 in Mongolia	6.4%	Highest tertile (≥10.33) vs lowest (≤6.72) mmol/L	Percentage of MetS 17.1% vs 4.6%	Fasting plasma insulin is associated with MetS in farmers, but not nomads among the Mongolian population in China.	[27]
STOPP- T2D PSG* 2008	2003 Cross sectional	1453 8 th grade adolescents in the U.S.	9.5%	Highest quintile (≥39.1) vs lowest (≤17.0) μU/mL	OR (95%CI) 199.64(31.29- 1273.7) for MetS	The highest insulin quintile were almost 200 times more likely to be classified with the metabolic syndrome than participants in the lowest quintile.	[28]
Adam FM et al 2006	2005 Cross sectional	128 overweight/ obese in Indonesia	68.8%	Mean fasting insulin levels15.68±7.85 vs 3.16±2.53 (uU/ml) with 5 components vs 1 component of MetS.		There is a strong linear increase in fasting insulin levels with an increase of the number of metabolic syndrome.	[29]

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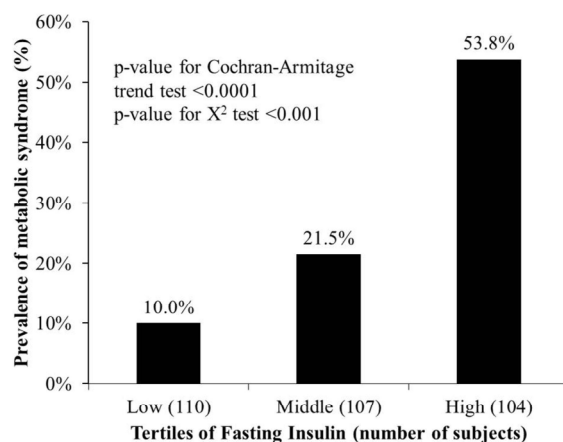


Figure 1 Prevalence of metabolic syndrome based on insulin levels. A linear increasing trend across insulin tertiles.

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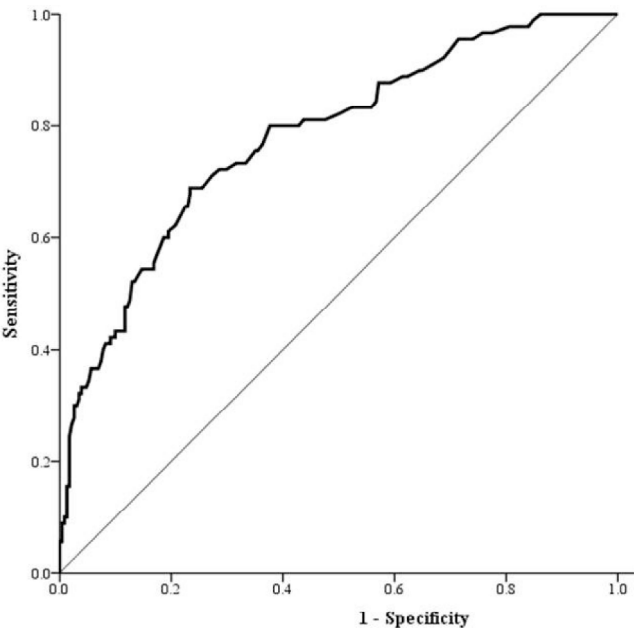


Figure 2. ROC curve for insulin as a biomarker of metabolic syndrome. AUC 0.78. FI 7.35 μ U/mL (sensitivity: 0.69; specificity: 0.77).

Figure 2. ROC curve for insulin as a biomarker of metabolic syndrome. AUC 0.78. FI 7.35 μ U/mL (sensitivity: 0.69; specificity: 0.77).

297x209mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	P2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	P2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any prespecified hypotheses	P2, P4
Methods			
Study design	4	Present key elements of study design early in the paper	P4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P4, 5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	P4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	Nil
Study size	10	Explain how the study size was arrived at	Nil
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	P6
		(b) Describe any methods used to examine subgroups and interactions	P6
		(c) Explain how missing data were addressed	Nil
		(d) If applicable, describe analytical methods taking account of sampling strategy	Nil
		(e) Describe any sensitivity analyses	Nil
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Nil
		(b) Give reasons for non-participation at each stage	Nil
		(c) Consider use of a flow diagram	Nil
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P8
		(b) Indicate number of participants with missing data for each variable of interest	Nil
Outcome data	15*	Report numbers of outcome events or summary measures	P6-9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P7
		(b) Report category boundaries when continuous variables were categorized	P8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Nil
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Nil
Discussion			
Key results	18	Summarise key results with reference to study objectives	P7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P17
Generalisability	21	Discuss the generalisability (external validity) of the study results	P18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.